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Journal of Dental Research: The Association's official publication. See register of officers and editors on page 2 of cover.

NOTICE

The next General Meeting of the International Association for Dental Research will be held in Chicago, Illinois at the Drake Hotel, June 21 and 22, 1947.

INFORMATION FOR PROSPECTIVE CONTRIBUTORS, SUBSCRIBERS, OR ADVERTISERS

The JOURNAL OF DENTAL RESEARCH was dedicated by its founder to the dissemination of knowledge pertaining to the teeth and mouth, and to their relations to the body as a whole. It publishes original investigations in these and related fields. Articles are accepted only on condition they are contributed solely to this JOURNAL. Articles appearing in other domestic or foreign periodicals will not be reprinted in the JOURNAL except in extraordinary cases. The JOURNAL will supply a limited number of half-tones or zinc etchings without cost to the author. References to the literature must conform to the following style:

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2. (To a book)—Doe, J. A., *The Dental Practice* (Chicago, Smith: 1930), p. 114.

See McBride, *J. D. Res.*, **16:** 537, 1937.

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THE EDITOR'S VIEWPOINT

McClure and Hewitt (p. 441) present a significant pilot study on penicillin and caries. Their rat caries experiments indicate a striking decrease in caries occurrence in penicillin consuming animals and suggest the need for more extensive studies of the type being carried on by Thomas J. Hill in humans. The question that will be raised is, "Will the use of penicillin in caries control aid in establishing penicillin resistant strains of pathogenic organisms and thus decrease or nullify its effect against deadly cocci?"

Weinmann (p. 509) shows that rachitogenic diets produce changes in the mandibular condyle of the white rat analogous to those observed in the cartilages of long bones. The possible effect on tooth eruption is discussed.

Dummett (p. 421) gives a very complete review of the subject of oral pigmentation in the Negro and in a further study, correlates oral and skin pigmentation. In view of the common misconceptions of all oral pigmentation as pathologic, the thesis presented is important to any dentist or physician who examines the oral tissues of Negroes or of any individuals suspected of heavy metal intoxication.

Restarski (p. 433) gives some interesting data on the effect of refrigeration as an anesthetic agent. This is a different principle from the chilled bur described previously and involves the cooling of the inferior dental nerve to as low as 11.5° C. Complete anesthesia was evidenced in 63% of cavities prepared in teeth anesthetized by nerve refrigeration. The tradition set by the immortals, Wells and Morton, is carried on as dentists continue the search for the ideal pain eliminator.

Keyes (p. 469) applied NaF solutions topically to hamsters with apparently slight protection against caries. A possible sex difference in protection as well as susceptibility to caries was noted. This work serves to emphasize the need for more intensive studies on fluoride-caries relationship and is evidence that the use of fluoride in caries prophylaxis is not justified as a routine procedure. We still believe that any known safe caries inhibitors (including fluorine) should be utilized in dental practice with the understanding that there are still many questionable features and such practical application is "experimental" or "demonstrational."

Permar, Kitchin and Robinson (p. 475) report the variations on counts of lactobacilli made from single specimens of saliva. The wide variation that they found may throw doubt on the value of any salivary lactobacillus count. However, trends were observed in long terms studies of individuals which indicate that the counts are still valuable measuring devices. The warning is against conclusions as to the value of anticarious agents based on a few isolated salivary lactobacillus counts. Efforts should be continued toward refinement of the present technic.

Weisberger (pp. 487 and 491) gives two further reports on his studies of saliva. He found that saliva could be substituted for tryptophane and to a much lesser degree for casein hydrolyzate in a synthetic media for *L. acidophilus*. He also observed that glucose, in saliva, favors proteolysis rather than deamination. The basic studies should be of interest to all caries investigators.

Bauer (p. 501) gives his findings in cases of acute secondary neutropenia. The dangers of this disease are ever present, some individuals being sensitive to arsenic, others to aminopyrines and, apparently, a few to "D.D.T." Bauer's observations clearly indict the tissue next to the gingival sulcus as the point of origin of oral necrosis. The fact that this is also the starting point for most Vincent's (necrotic) gingivitis is highly significant.

Irons' paper (p. 497) is more basic than most of our contributions. Dr. Irons, an assistant professor of operative dentistry,

is carrying on his investigations in the fertile but relatively uncultivated field of chemical pathology. The work reported to date is of interest to oral investigators and others working in the field of regeneration, repair and neoplasms.

Orland (p. 445) has given a careful description of the hamster and its tooth development. The molar lesions they develop are also given due consideration. This study, based on 682 animals, is essential to all work on experimental dental caries in hamsters. In a study of the oral bacterial flora of the Syrian hamster (p. 455) the association between lactobacilli and streptococci and the beginning of caries was noted. Numerous diets with different vitamin contents were utilized but "no clear evidence of a direct relationship between number of microorganisms isolated and vitamin content of the various diets." Orland's findings should be compared with those of Keyes (*J. D. Res.* 25: 341, 1946 and 25: 469, 1946).

H. B. G. R.

Rabkin (p. 513) discusses oral pathology of sheep and goats, an infrequent type of discussion in American dental literature. This presentation shows that affiliation with a research institution is not essential for scientific investigations. Dr. Rabkin used material available to him from abattoir. That it is dangerous to transfer evidence from lower animals to man, without careful evaluation of inherent variations of anatomy, physiology and pathology, is demonstrated in an exaggerated form by the evidence given.

Our alert former President, W. D. Armstrong, picked up a glaring error in the "Editor's Viewpoint" for October (p. 297). We erroneously reported that Greenwood, Blayney, Skinsnes and Hodges had noted significant effects on the serum Ca and P, Hb, blood coagulation time and growth of puppies fed various forms of fluorine. Actually, Greenwood, Blayney, Skinsnes and Hodges found no significant changes in the serum Ca and P, Hb, blood coagulation time and growth. We apologize to the authors and thank Dr. Armstrong, first for noting the error and secondly for reading the "Editor's Viewpoint."

H. B. G. R.

PHYSIOLOGIC PIGMENTATION OF THE ORAL AND CUTANEOUS TISSUES IN THE NEGRO[1]

CLIFTON O. DUMMETT, D.D.S.

Department of Oral Health, Meharry Medical College, Nashville, Tenn.

The components of the Negro population are in the majority of cases a sharply defined element of the United States population. First introduced to this country late in the sixteenth and early in the seventeenth centuries, this group of people has continued to increase until at the present time, the Negro population is the largest of America's minority groups. From the very beginning there has been both legitimate and illegitimate mating between the Negro and white groups, with the result that a large percentage of the Negro population contains some degree of intermixture. As a result of these unions, there have been produced a large number of individuals with obviously Caucasian characteristics, who however have been classified as Negroes because of a peculiar yet prevalent custom that includes as Negroes all individuals having a 'single drop' of Negro blood. Intermixing is likely to continue until possibly the whole Negro population of the United States will be involved.

There is a great amount of variation in the products resulting from these and other racial intermixtures. The variations are most easily discernible in the anatomical characteristics of the resultant group. Particularly obvious among these is the characteristic of skin color which in the United States is the physical trait by which intermixture is usually determined. When it is realized just how great are the variations in shades of complexions that can be seen in individuals who are classified as Negroes, and also in some individuals who are classified as belonging to other racial groups, and when it is noted just how often there is an overlapping of colors between the members of these different groups, it is to be wondered just how accurate a criterion of racial grouping is the color of the skin.

The uncertainty of the skin color that may be evidenced in the off-spring of families with an intermixture of white and Negro blood has made for the exceedingly great interest in the question of inheritance of skin color. Some investigators indicate a belief in the inheritance of skin color in humans by blending. Others indicate that there is the probability of segregation in color inheritance. It is probably true that a combination of many factors might account for inheritance of skin color. There are however a large number of details that must be

[1] Presented in part at the 24th General Meeting of the International Association for Dental Research, Kansas City Mo., March, 1946, (*J. D. Res.* **25**: 155, 1946) and submitted in part as a thesis for a specialist license in Periodontia to the Tennessee State Board of Dental Examiners, June 1946. Received for publication June 17, 1946.

The author wishes to express sincere appreciation to the Viking Fund, Inc. of New York, for making possible this publication.

fully worked out before a completely satisfactory solution to the problem can be presented.

THE NATURE OF PIGMENTATION

A study of the quantitative distribution of pigments in the normal skin by Edwards and Duntley demonstrated the existence of 5 cutaneous pigments. These were melanin, a derived material described as melanoid, carotene, reduced hemoglobin and oxyhemoglobin. In addition these investigators confirmed the idea that the colored races owe their characteristic colors only to variations in the amount of melanin and the derived pigment melanoid.

Melanin is a product of cell activity and is a complex organic dye which varies in color from bright red and reddish yellow through light and dark brown to black. It can be chemically isolated from the tissues and is known to have a high molecular weight. It is formed in the protoplasm of certain cells called melanoblasts or pigment-forming cells. Melanin cells are normally present in the unpigmented skin as clear cells which under the influence of certain stimuli (ultraviolet rays, radium or roentgen rays) enlarge and form melanin. The discovery of the now famous 'dopa reaction' by Bloch is regarded as being the most significant single step in the solution of the pigment problem. The reaction is a specific indicator of the pigment producing power of a cell and consists of the production of a diffuse blackening of the entire cellular protoplasm as a result of the chemical reaction between 3,4-dioxyphenylalanin (dopa) and an oxidizing factor dopaoxydase (Bloch) in the melanoblasts with the production of a black melanin-like substance. The reaction is found only in those cells which are capable of producing pigment. This intrinsic source of pigmentogenesis should be differentiated from cases in which pigment is produced by extraneous agents such as carbon, coal, iron and the heavy metallic drugs. In addition, Edwards and Duntley draw a distinction between primary and secondary normal melanin pigmentation. The former is controlled by racial and constitutional factors while the latter varies with exposure to sunlight and disappears after removal of this stimulus.

The various shades of pigmentation in the skins of Negroes and the brownish tint of the skin of dark complexioned Caucasians and of some pathologically pigmented persons are attributable to an increased quantity of epidermal (ectoderm) melanin and in a much lesser degree to an increase in dermal (mesoderm) melanin. Becker claims that the dermal pigment plays the more important role in excessive pigmentation in some cases of tar and arsenical hyperpigmentation, in cases of incontinentia pigmenti (Bloch), and in Mongolian spots and blue nevus (Jadassohn). In addition to this a bluish color is more often seen in the skins of animals (e.g. ape). This is attributable to the large numbers of dermal melanoblasts found to be present in these cases.

CUTANEOUS AND EXTRACUTANEOUS PIGMENTATION

In man, cutaneous pigmentation is found over the entire body surface and is dependent on a variety of factors, principal among which are the thickness and

transparency of the epidermis, the blood content of the superficial vascular bed and the amount of pigment. In white individuals in whom lesser amounts of skin pigment are found, there occurs a regional variation from greatest to least as follows: nipple and areola, navel, genitalia, dorsal surface of the forearm, hypogastrium, axilla, epigastrium, and finally the flexor surfaces of the extremities and palms and soles. There is a greater amount of pigmentation in the skins of Negroes. Niedelman states that concentration of pigment in different areas of the body varies in the Negro race in the same manner as in the white race. It has been my observation that in some Negroes conforming to shades of 'very dark' and 'light brown' (see classification which follows) there is a tendency for the concentration of skin pigment to be relatively uniform, no one region being more obviously 'clinically pigmented' than another. Negroes of 'brown' and 'fair' shades seem more nearly to conform to regional color variations.

Extracutaneous pigmentation is found in the mucous membranes of ectodermal origin. Among these are included the inner surfaces of the male and female prepuce, and the superficial part of the lacteal ducts of the nipple, the anal canal, the pharynx and oral tissues.

ORAL PIGMENTATION

In the case of the oral tissues, pigmentation is observed in a large number of individuals who evidence no pathosis on careful examination. In an examination carried out on his series of 220 Negro patients, Monash states that the normal regional variation in oral pigment from greatest to least is as follows: gums, cheeks, hard palate, tongue, soft palate, and floor of the mouth.

Oral pigmentation is by no means confined to members of the Negro race. It has been reported in the French, Filipino, Algerian, Arabian, Chinese, East Indian, German, Gypsy, Roumanian and natives of Asia minor. In addition to these, Monash reported the occurrence of oral pigmentation in members of the following groups: Italian, Jewish, Greek, Porto Rican, Syrian, Peruvian, and Ceylonese. Physiologic oral pigmentation is therefore not limited to any one race although it does appear to be a common characteristic of the darker races. Becker has demonstrated that although clinically absent some degree of epidermal pigmentation was seen microscopically in the normal buccal mucosa of 74% of 53 unselected autopsies. All of Becker's cases were white and mostly of medium complexion. As a result of these studies he concluded that pigmentation of the buccal mucosa seemed to be normal.

The pigmentation of the oral tissues is comparable to the pigmentation of the skin. There is a great similarity between the histologic structure of the skin and that of the gingiva, which as has previously been mentioned, is one of the more accessible and usually one of the more heavily pigmented of the oral tissues. The skin consists of two layers—a superficial epidermis of stratified squamous epithelium and an underlying dermis. The epidermis has two layers, the stratum malpighii and the stratum corneum.

In the stratum malpighii there is present melanin pigment which accumulates as fine granules within the cells of this region, particularly in the basal layer. As these cells move toward the stratum corneum the granular pigment disappears

so that only a diffuse coloring is observed in the stratum corneum. In the Negro, skin pigmentation is due to the great amount of pigment in all the layers of the epidermis. In the dermis there are found some melanin-containing cells which are called dermal chromatophores. However the role that these cells play in determining skin color is rather indefinite.

In the gingiva there is present epithelium which is of a stratified squamous variety. Hornification of the gingiva is a usual though not constant characteristic. Located beneath the epithelium is the lamina propria or connective tissue region which is comparable to the dermis or corium of the skin. In the basal layer of the stratified squamous epithelium are found cells which may or may not contain pigment granules (melanin). When this pigmentation is found in Negroes, then it is an indication that there are excessive numbers of the pigment cells in this layer. Melanoblasts have not been demonstrated in the lamina propria of the gingivae.

It may therefore be said that these black pigments are normally found in the skin and gingiva in addition to being found in the other oral tissues, the iris and choroid coat of the eye, the cerebral meninges and the hair. They are found in all human beings with the exception of albinos in whom all pigment is lacking. This condition of albinism is characterized by a prenatal partial or complete lack of pigment. It is believed that the condition is familial and hereditary. The skin in the involved areas is pink due to the blood which is unrestrictedly reflected through the epidermis, the hair is white and the person suffers from photophobia due to the lack of protection afforded by ocular pigment. A clinical examination of the oral tissues reveals a complete absence of melanin pigment.

RELATIONSHIP OF ORAL TO CUTANEOUS PIGMENTATION

As has been previously stated, it seems that physiologic pigmentation is a characteristic of the darker races although it is not limited to any one race. Inasmuch as microscopic examination of areas of pigmentation of the oral tissues shows a picture identical with that of pigmentation of the skin, it is to be wondered whether pigmentation of the oral tissues is constantly associated with cutaneous pigmentation. Monash examined 220 Negroes of varying shades of pigmentation, and found that there existed pigmented spots in the oral mucosa in 95% of the cases. Of the 5% which showed no pigmentation all were of the lighter group. However the number of cases wherein individuals who may be classified as being 'highly pigmented' (very dark and brown) show no clinical evidencss of oral pigmentation, is large enough to question the usually assumed unvarying constancy of association between oral and cutaneous pigmentation.

In the following study 636 Negroes were examined to find out the relationship between oral tissue pigmentation and skin color. The oral tissues were examined under intra-oral illuminators while the skin was examined in bright daylight. Inasmuch as there was no attempt to compare the shades of color of the oral tissues and the skin, the errors in these different methods of examination were not significant.

The oral tissues that usually evidence melanogenous pigmentation are the gingivae, buccal mucous membranes, tongue, hard palate, and lips. Less fre-

quently and obviously, pigmentation is found in the floor of the mouth and in the soft palate. In this study attention has been directed to the type, character and region of pigmentation occurring in the gingivae, buccal mucosa, tongue and hard palate. The type of pigmentation was noted as follows: Absent, medium and heavy; the character was described as mottled, partial or complete; and the exact region in which pigmentation occurred was noted.

Attention is called to the very valuable and important study on the determination of black pigmentation in the skin of the American Negro by Todd and Van Gorder, in which an exceedingly high degree of accuracy in quantitative estimation of skin color was achieved by the use of modifications of the Bradley color top. The color top was also employed in Monash's observations on oral pigmentation in 220 Negroes. Such relatively accurate methods of determination of percentage of black skin pigment were not used in this study.

Under the heading Complexion there were 4 divisions: 1. Very dark; 2. Brown; 3. Light brown; 4. Fair.

This arbitrary classification is but an approximation of the colors of the skin and does not pretend to include all the various shades of complexions that are found in members of the so-called Negro race, but it was found to be satisfactory for the purposes of these observations. Those cases in which the complexion was difficult to classify because of a possible conflict in color determination were not used. The general complexion of the skin in the lower one-half of the face (from the tip and base of the nose to the mentum) was used as a basis of comparison with the color of the oral tissues. It is true that the color of this portion of the anatomy is often of a shade different from that of some more protected surface. Exposure to the action of the sun's rays resulting in tanning and the production of freckles, wind, temperature changes, various cosmetics and other irritating factors usually result in an increase in the amount of melanin and a darkening in color of the part involved. The intraoral tissues are also exposed to some of these irritating factors which may influence their color.

Through the courtesy of the Department of Obstetrics of Meharry Medical College, it was possible to examine several newborn infants to determine the oral tissue color at this level of development. All other cases seen were patients at the dental clinic of the college where the examinations were made.

Results of Observations

In this study observations were made on:

1. 65 Negroes of 'very dark' complexion.
2. 283 Negroes of 'brown' complexion.
3. 127 Negroes of 'light brown' complexion.
4. 94 Negroes of 'fair' complexion.
5. 67 Infants.

Oral Pigmentation in 'Very Dark' Negroes

In Class I, 65 individuals of 'very dark' complexion were examined. The ages of these individuals ranged from 17 to 48 years. 49 persons possessed a heavy diffuse type of pigmented gingivae, 11 medium pigmented gingivae, and

in 5 cases pigmentation was clinically absent. The pigmented areas varied somewhat in appearance with their location. In general it may be stated that the amount of pigmentation appeared to be more concentrated in the anterior portion of the mouth, and tended to diminish in the posterior regions. The lingual surfaces of the gingivae were less pigmented than the labial and buccal surfaces but the quantitative variations in the regions of pigmentation followed the same general pattern as the latter surfaces.

The general pigmentation appeared to be a heavy deep purplish blue band extending from the gingival crest practically all the way up to the muco-labial and muco-buccal folds (*fig. 1*). There were a few cases in which the pigmentation did not include about 3mm. of the crests of the gingival tissue. This tissue was pink and could be classified as clinically non-pigmented (*fig. 1 A*). Some investigators believe that this lack of pigmentation at the gingival crest is characteristic of Negroes, especially in those cases where other gingival pigmentation is present. It is claimed further that a darker color change in the gingival crests of such cases is the important point of diagnosis of the pigmentation of bismuth or other metallic poisons. As a result, many cases have been diagnosed as bismuth pigmentations solely on the basis of the presence of a complete pigmentation up to and including the gingival crests. This has not been in accord with the author's observations. The absence of gingival crest pigmentation in the presence of other pigmentation is to be considered as one among the many variations of pigmentation that may be manifested in these tissues.

Oral examinations of over 200 Negro individuals undergoing anti-luetic therapy over varying periods of time, from the initiation to the completion of treatment indicated that, regardless of the presence or absence of pigmentation in the gingival tissues, there was the tendency towards a characteristic staining of the free margins of the gingivae in individuals who had been subjected to bismuth injections[2]. In those cases of severe bismuth stomatitis there was an extension of the staining to involve tissues other than the free gingival margins. In the cases in which an original pigmentation existed, there occurred later a gray-white or purple-white color that seemed to be superimposed on the purplish-black base. It was this color change that formed a basis of diagnosis of bismuth intoxication in these specific cases rather than the presumed darker color change in those gingival crests which originally lacked the pigmentation that was present in the remaining gingival tissues.

The number of cases in this group in which pink gingivae were found (*fig. 2*) substantiate the author's previous criticisms relative to the unvarying constancy of association between oral and cutaneous pigmentation. In these instances the gingivae were of a uniform light pink color, translucent, and in addition they possessed a dull stippled appearance. Medium pigmented tissues were those that seemed to be mixtures of the pink and highly pigmented tissues.

Examinations of the mucous membranes showed that in the majority of cases

[2] These studies on bismuth pigmentation in mouths with already present oral pigmentation are in progress and will be reported on in a subsequent article.

these tissues were clinically pigmented whenever the gingival tissues were similarly affected. In a few cases the mucous membranes were pink while the gingivae were pigmented, but there was not a single instance in which pigmented mucous membranes accompanied pink gingivae. When occurring, the pigmentation tended to be diffuse, spreading over the entire surface, and varying in severity from a deep purplish color to a very light grayish color.

Some pigmentation of the tongue was seen in 15 cases and these were all cases with heavily pigmented gingivae. The pigmentation occurred as light brownish or more heavily pigmented single or multiple spots or plaques of varying size on the dorsum, tip or sides. There was no tendency for the pigmentation to be symmetrical, and in this respect, the tongue was unique inasmuch as all the other tissues examined evidenced a symmetrically bilateral pigmentation.

In the case of the hard palate the incidence, type and severity of pigmentation was directly parallel to that of the labial and buccal gingival tissue. This is to be expected inasmuch as the lingual gingival tissue is both a continuation and a part of the hard palate. On the whole, the pigmentation of the tissues comprising the hard palate appeared to be somewhat less quantitatively than that of the labial and buccal gingival tissues.

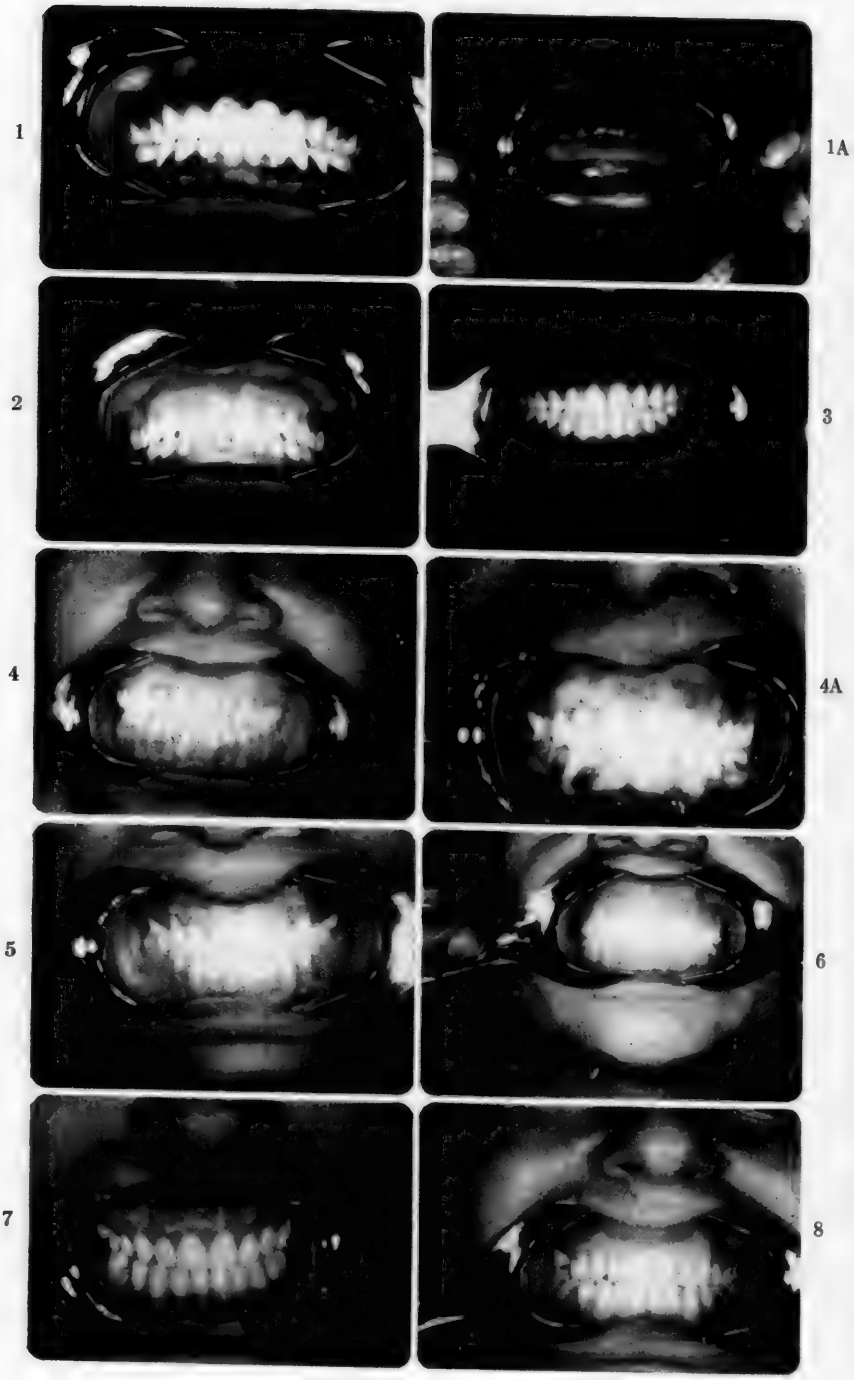
Oral Pigmentation in 'Brown' Negroes

In Class 2, 283 individuals of 'brown' complexion were examined. The ages of these people ranged from 15 to 54 years. 141 of the cases showed a heavy diffuse type of gingival pigmentation (*fig. 3*), 67 showed a medium gingival pigmentation, and in 75 cases pigmentation was clinically absent (*fig. 4*). As can be seen there were relatively more cases of clinically non-pigmented gingivae here than in Class I. In addition there were a few cases where there existed a unilateral and asymmetrical type of pigmentation (*fig. 4 A*). This was in contrast to the large majority of cases where the tendency was for the pigmentation to be symmetrically bilateral.

Forty-seven of the cases examined in this class showed mucous membranes with some degree of pigmentation. In these cases the pigmentation appeared as a symmetrical diffuse or mottled gray pink color that seemed to be superimposed upon a deep seated light brown base. These tissues were translucent, smooth and shiny. Monash states that the anterior third of the cheek is the area of predilection.

In 71 cases, pigmentation of the tongue was observed. Generally, the pigmentation occurred as light brown or purple spots, confined to the fungiform and filiform papillae, although in a few cases there were present pigmented plaques approximately 1 cm. square. As in Class I, the pigmentation was asymmetrical.

Pigmentation in the hard palate very closely paralleled that on the labial and buccal surfaces in incidence and type, even though there appeared to be a generally lesser amount of pigment in these tissues.



FIGS. 1–8

Fig. 1. Negro female aged 21 years of 'very dark' complexion with diffuse heavily pigmented gingival tissues. The buccal mucous membranes are of a grayish purple color, the tongue is light pink with pigmented macules on the tip and sides, and the hard palate is heavily pigmented especially near the cervical portions of the teeth. The lips are heavily pigmented.

Fig. 1A. shows a Negro male aged 38 years of 'very dark' complexion with edentulous mouth showing a heavy purplish black pigmentation. In the crest of the upper arch can be seen a continuous pink ridge devoid of pigment. This would probably correspond to the pink gingival crest areas in those dentulous cases with other gingival pigmentation. The mucous membranes are of a bluish gray color, the tongue reddish pink, and the hard palate a purplish black color, greatest near the linguo-gingival tissues. The outer borders of the upper and lower lips are brownish black in color.

Fig. 2. Negro female aged 32 years of 'very dark' complexion with pink gingivae completely devoid of any clinical evidences of pigmentation. The mucous membranes of the lips and cheeks are pink, the tongue reddish pink, the hard palate pale pink and the outer borders of the lips are highly pigmented.

Fig. 3. Negro female aged 22 years of 'brown' complexion with a diffuse type of heavy purplish brown pigmentation of the gingivae. The buccal mucous membranes are slightly pigmented appearing somewhat grayish in color. The tongue is pink, the hard palate is heavily pigmented especially near the gingival tissues, and the outer borders of the lips are highly pigmented.

Fig. 4. Negro female aged 31 years of 'brown' complexion with light pink gingivae lacking clinical signs of pigmentation. The mucous membranes are pink, the tongue reddish pink and the hard palate a pale pink. The outer borders of the upper and lower lips are of a medium brown pigmentation.

Fig. 4A. Negro female aged 24 years of 'brown' complexion with asymmetrical pigmentation of the gingival tissues. The left portion of the upper gingival tissues are of a brownish pigmentation while the right portion has a pink area surrounded by a lighter brownish border. In the lower arch there is an area of deep purplish brown pigmentation to the mesial of the right cuspid. The whole lower anterior region is pink, while the posterior region is of a light brownish color. This may be considered a form of 'piebald' gingival tissue. The mucous membranes are pink and the tongue reddish pink. The hard palate is pink and shows no evidence of pigmentation near the gingival tissues. The outer borders of the lip are of a brownish color.

Fig. 5. Negro female aged 18 years of 'light brown' complexion with gingival tissues of light brownish pink pigmentation. The buccal mucous membranes are pink and the tongue reddish pink. The hard palate is of a light brown pigmentation especially near the lingual gingivae, and the outer borders of the upper and lower lips are brownish pink.

Fig. 6. Negro female aged 22 years of 'light brown' complexion with gingival tissues of a reddish pink color without any clinical evidences of pigmentation. The mucous membranes are pink, the tongue reddish pink and the hard palate light pink. The outer borders of the upper and lower lips are slightly pigmented.

Fig. 7. Negro female aged 19 years of 'fair' complexion with gingival tissues having a slight brownish pink pigmentation in the upper and lower anterior areas. The greatest concentration of pigment is in the cuspid area. The buccal and labial mucous membranes are light pink and the tongue reddish pink. The hard palate is light pink and contains a line of light brown pigmentation around the linguo-gingival portions of all the teeth. The lips are reddish pink in color.

Fig. 8. Negro female aged 27 years of 'fair' complexion with gingival tissues and mucous membranes of light pink color. No clinical evidences of pigmentation are present. The tongue is reddish pink, the hard palate light pink, and the outer borders of the upper and lower lips are reddish pink.

Oral Pigmentation in 'Light Brown' Negroes

In Class 3, 127 Negroes of 'light brown' complexion were examined. The ages of these people ranged from 16 to 38 years. *None* of the cases showed a heavy diffuse type of gingival pigmentation. 73 showed some degree of gingival pigmentation (*fig. 5*) and in 54 cases pigmentation was clinically absent (*fig. 6*) When present, the pigmented areas appeared as flat light brown irregularly circular areas which occupied approximately the central portion of the gingival surface. It is of interest to note that in none of the cases in this group was there found a color approximating that of the greatest amount of pigment found in cases in Class I.

Only 22 individuals in this group possessed mucous membranes that were clinically pigmented, and in no case did the intensity exceed a deep grayish pink. In none of the cases examined was there any evidence of pigmentation of the tongue.

The hard palate was pigmented in 45 cases. This was generally a lesser amount of pigmentation in this region than was found on the labio and bucco gingival surfaces.

Oral Pigmentation in 'Fair' Negroes

In Class 4, 94 Negroes of 'fair' complexion were examined. The ages of these people ranged from 17 to 48 years. Of the 94 cases examined, only 43 showed any clinical evidence of gingival pigmentation. In the majority of cases the pigmentation was slight and appeared as flat very light brownish areas somewhat elliptical and with the deepest concentration of pigment approximately in the center of the gingival surface (*fig. 7*). In 51 cases clinical pigmentation was absent (*fig. 8*).

Four cases showed slight grayish pigmented mucous membranes. In no case did the tongue show any evidence of melanin pigmentation.

Thirty-three cases showed a very slight pigmentation in the gingival portion of the hard palate near to the amelocemental line.

Oral Pigmentation in the Newborn

Oral examinations made of new born infants showed that in these cases gingival pigmentation was present as early as 3 hours after birth. In many cases pigmentation was observed only on the gingival surfaces. This is in accord with Monash's observations that clinical pigmentation develops first on the gingival surface. In a total of 67 infants, only 1 was classified as fair. In this case there was no evidence of gingival pigmentation. In the 23 infants classified as 'light brown', only 8 showed some slight evidence of gingival pigmentation. The large majority of 'brown' and 'very dark' infants showed gingival pigmentation ranging from a very light brown in most cases, to a purplish brown color. In an examination of 18 infants in the first week of life, Monash found some pigmentation in 6 of these infants. He states that there is a relative paucity of both skin and oral pigmentation at birth, but that increased skin pigmentation occurs during the first few years of life and is accompanied by increased oral pigmentation.

SUMMARY AND CONCLUSIONS

Clinical observations indicate that in Negroes with very dark skin colors, there is a strong tendency for the oral tissues to be highly pigmented. As the skin color lightens, there is less of a tendency for the oral tissues to be pigmented, so that in very light Negroes, obviously pigmented oral tissues are seldom observed. It is no rarity, however, to find in Negroes of very dark skin colors oral tissues that give no clinical evidences of melanin pigmentation. Conversely, some degree of pigmentation has often been found in the mouths of many members of the Caucasian group.

Custom, ignorance and superstition have often made for occasional undesirable psychological reactions against the presence of this oral pigmentation. Many have claimed that its appearance is unesthetic and unsightly with the result that attempts have been made to eliminate it when present. Hirschfeld is credited with making some of the initial attempts at a method of eliminating gingival pigmentation. Attention is called to *fig. 3*. The harmonious blending of gingival and cutaneous pigmentation produces an unmistakable esthetic effect.

In the examinations carried out in this series of adult patients, the following was the order of frequency of occurrence of pigmentation: Gingival tissues in 67% of all cases, hard palate in 61%, mucous membranes in 22%, and the tongue in 15%.

The skin and the oral tissues are the 2 tissues in which color characteristics can be most easily noted. An unvarying constancy of association of the presence of pigmentation in these tissues has been assumed, and used as a basis of racial grouping. The inconsistency of this presumed association together with the unlimited variations in the manifestations of pigmentation when the latter does occur indicate that any identification of racial grouping solely on the basis of color changes would be very inaccurate and unscientific. The presence of oral pigmentation may be employed as a basis for the diagnosis of various dental, systemic and more recently, various occupational diseases. The presence of sub-gingival calculus and its production of a darkened effect on the gingival tissues, the generalized pigmentation of the oral mucosa in Addison's disease, and the oral tissue discolorations subsequent to exposure to bismuth, lead and mercury—are just a few pertinent examples of cases in which the pigmentation is of diagnostic importance. However, further study on this subject is needed in order to furnish adequate information about the early recognition of many other pathological conditions so that they may be treated or prevented at the earliest opportunity.

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ANESTHESIA INDUCED BY LOCAL REFRIGERATION OF THE JAWS[1, 2]

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Alleviation of pain in dental operations by using local refrigeration (this does not imply freezing) was reported as early as 1863. At that time, Oudry (1) described an apparatus for cooling the teeth prior to extraction. Chaydeane and Black (2) in 1937, employing a method similar in principle to that used by Oudry, conducted experiments on dogs and two human subjects. They used small hollow metallic applicators held firmly against the gingival tissues at the level of the root of the tooth. Brine chilled at 33°F. was circulated through the applicators. They found that dental anesthesia, as determined by the reaction of the subjects to an electric pulp tester, was effected in "less than a minute."

In a preliminary study at this Institute (3), histologic investigation disclosed that continuous cooling of localized areas in the jaws of dogs, at 1–2°C. for as long as two hours, did not produce pathologic changes in the gingivae, teeth, or related structures.

In view of these results with dogs, it was decided to test the efficacy of refrigeration as a local anesthetic, by drilling and filling the teeth of human subjects. The preliminary findings were encouraging in that of twenty-two cavities prepared, fifteen operations were completely painless, while in seven, a mild degree of pain was experienced. Similar results were recently reported in clinical trials on nine patients at the School of Dental and Oral Surgery, Columbia University (4).

In the study reported here, additional trials on human subjects were made to determine more completely the effectiveness, limitations, and practicability of this type of local anesthesia and improvements in the refrigerating apparatus, particularly in the construction of gingival applicators. Additional temperature and histologic studies were carried out on dogs.

PROCEDURES

Refrigerating unit and applicators.—The cooling system used in this study was a thermostatically controlled Freon unit (*fig. 1*). The use of Freon was a great improvement over the ice-salt mixture employed in a previous study (3). The circulating refrigerant employed (alcohol, 35%, and water, 65%) was the same in both studies.

[1] The material in this article should be construed only as the personal opinion of the writer and not as representing the opinion of the Navy Department officially.

[2] Received for publication July 10, 1946.

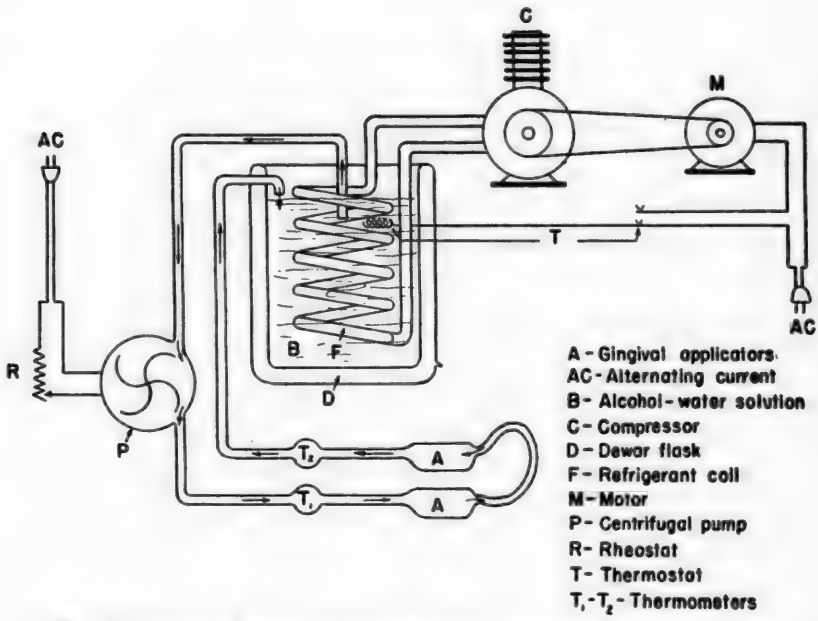

Fig. 1. Schematic diagram of thermostatically controlled refrigeration unit

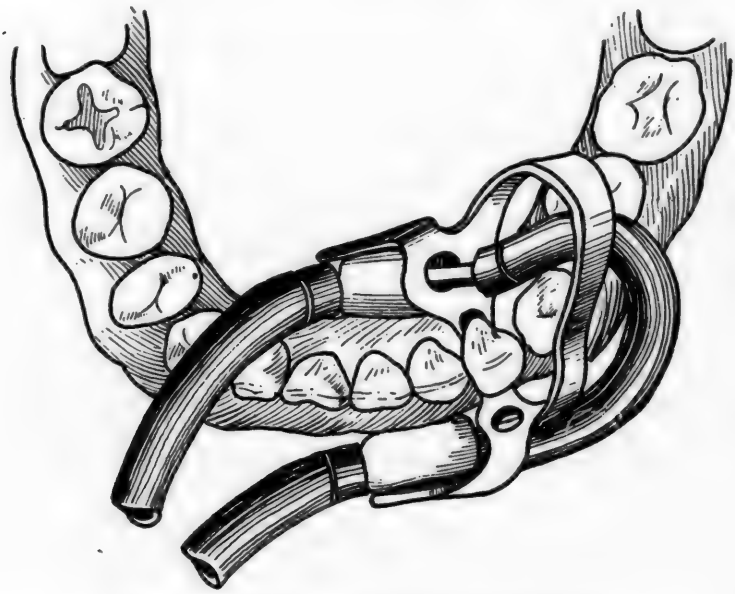

Fig. 2. Applicators in anterior mandibular position

The lowering of the temperature of the refrigerant passing through the applicators was controlled by regulating the speed of the centrifugal pump with a rheostat. This feature was important, since abrupt changes in temperature caused moderately severe pain in the jaws. This was true whether the temperature rose or fell. On discontinuing refrigeration, it was found that leaving the applicators in position on the jaw for about 2 minutes after stopping the circulation of the refrigerant, permitted sufficiently gradual warming to prevent pain.

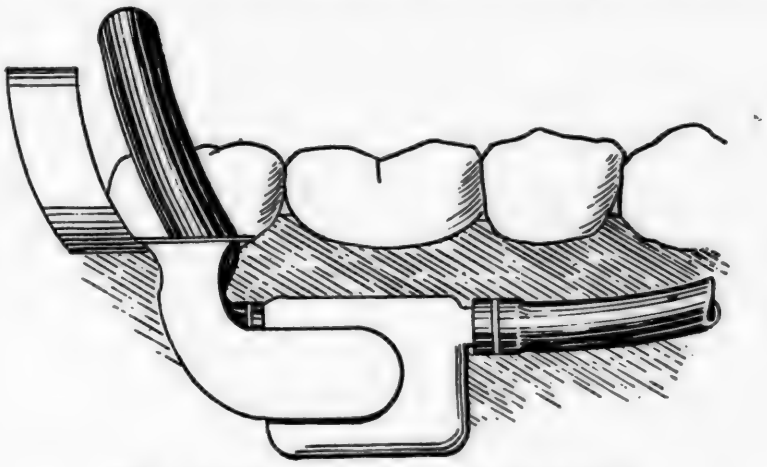

Fig. 3. External view of applicator in molar position.

An initial temperature of about 18°C. was comfortably tolerated. Thereafter, a temperature change of 5 to 6 degrees per minute could be tolerated with little or no discomfort. Using the present unit, it was possible for the subject to regulate the rheostat, and thus control the decrease in temperature at a comfortable rate.

The most difficult part of this problem has been, and still is, the construction of suitable gingival applicators that can be used interchangeably from one subject to another. Modifications were incorporated in the present design permitting better access to the various surfaces of the tooth to be drilled (*figs. 2 and 3*), but, in the majority of instances, it was practically necessary to construct a new applicator to fit each individual case.

The hollow metallic applicators were made of silver (*fig. 4*) to afford better conductivity. Retention of the applicators in the proper gingival position was attained by the addition of flat spring extensions, made of phosphor-bronze, to the wings of standard rubber-dam clamps.

Procedure on human subjects.—The effectiveness of the local anesthesia was tested by drilling and filling the carious teeth of naval personnel. As reported earlier (3), all cavity preparations were begun without refrigeration, and drilling was continued until a definite sensation of pain was experienced by the patient in order to present an adequate control. Once the coccurrence of pain was clearly

established, the applicators were placed in position in the gingival root area of the tooth and refrigeration was begun. When refrigeration (at 1–2°C.) had been in progress from 3 to 5 minutes, preparation of the cavity was resumed.

The major portion of the drilling of the cavity was now completed. It was during this portion of the drilling that observations on the degree of anesthesia were made. Following this, the applicators were removed and enough time (about 3 minutes) was allowed for normal sensation to return. Drilling of the cavity was again resumed, since pain may diminish or even disappear during preparation of a cavity. Only those cases in which pain recurred, after refrigeration was discontinued, were included in this study.

Dog experiments.—In a subsequent experiment, areas of the mandible of a dog, approximately 1 year old, were cooled at 0° to 1°C. for 2-hour periods. Four different areas were refrigerated, 2 weeks, 1 week, 2 days, and 15 minutes, re-

Fig. 4. Cross section view of applicator

spectively, before sacrificing the animal. By this procedure, it was hoped to detect early and late changes in the inferior alveolar nerves and in the nerves of the uncalcified tissues overlying the mandible.

Upon completion of the experiments, the dog was killed by an overdose of nembutal (pentobarbital sodium) given intravenously, and perfused through the aorta with 10% neutral formalin. The desired jaw sections were then removed and both inferior alveolar nerves carefully dissected out. Sections of the buccal and lingual uncalcified tissue were also taken from the surface of the mandible and preserved for study.

Since the sections in the earlier study (3) were stained with hematoxylin and eosin, it could not be determined whether changes occurred in the detailed portions of the nerves, particularly in the nerve fibers, axis cylinder, and myelin sheath. The specimens in the present study were treated with Bodian's silver stain for fibrils and axis cylinders, and with Spielmyer's myelin sheath stain.

In another experiment using 2 dogs, approximately 1 to 2 years old, temperature measurements were taken from the mandible during local refrigeration at 0° to 1°C. The measurements were made by copper-constantan thermocouples, placed in the various portions of the mandible, and a Leeds-Northrup potentiometer. Measurements were taken from the following areas of the mandible: (a) at the root end of the tooth adjacent to the inferior alveolar nerve; (b) at the

junction of the periosteum and bone; and(c) at the surface of contact of the refrigerating applicator and mucous membrane.

For experiment, the animals were anesthetized with nembutal by intravenous injection of 0.5 ml/kg. of body weight. A thermocouple was then placed in the area of the inferior alveolar nerve through a hole drilled axially in a molar in which the pulp canal was sufficiently enlarged. Once the thermocouple and gingival applicators were in position on the jaw, refrigeration at 0° to 1°C. was begun and the temperature read on the potentiometer. Temperature measurements were also taken from the other areas of the mandible by a similar procedure.

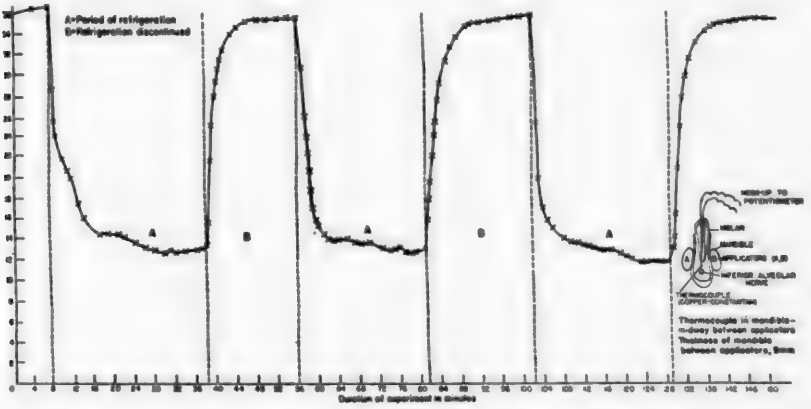

Fig. 5. Temperature measurements taken from the mandible of a dog, using copper-constantan thermocouple placed near the inferior alveolar nerve. During local refrigeration (temperature of refrigerant at applicators 0–1°C.)

RESULTS

Temperature measurements in the mandibles of dogs.—The lowest temperature attained in the area of the inferior alveolar nerve was approximately 11.5°C. (thickness of jaw between applicators was 9 mm.). Repeating the same experiment using a dog whose mandible was 16 mm. thick, a low temperature of 13.6°C. was reached. The most rapid fall, a decrease from approximately 37° to 15°C., took place within 7 minutes after refrigeration was begun (*fig. 5*). The subsequent drop in temperature was considerably slower (approximately 4.5°C. in 16 minutes). Upon discontinuing the circulation of the refrigerant, a rapid rise in temperature was noted (from approximately 13.6° to 34.6°C.) within 6 minutes.

The lowest temperature reached in the area between the bone and periosteum was approximately 7.5°C. (*fig. 6*). At the contact surface of the mucous membrane and the applicator, the lowest temperature registered was about 2.8°C. (*fig. 6*). In general, the decrease and return to normal temperature followed the pattern observed in measurements taken at the inferior alveolar nerve.

Histologic studies of nerves.—There was no evidence of injury in the axis cylinders, nerve fibers, nor in the myelin sheath of the inferior alveolar nerves, or the nerves of the mucous membrane and periosteum, following refrigeration of localized areas in the mandible at 0°–1.5°C. for 2-hour periods.

Results of trials with human subjects.—Fifty-two cavities were prepared for filling in 33 individuals (Table I). In 34 instances, complete anesthesia was obtained; in 13, very mild to moderate pain was experienced; and in 5 cases there was little or no anesthesia. The failure, in some instances, to obtain adequate anesthesia cannot be satisfactorily explained at this time. It may be due to improper adaptation of the applicators to the jaw, or possibly to the fact that the area refrigerated was too small.

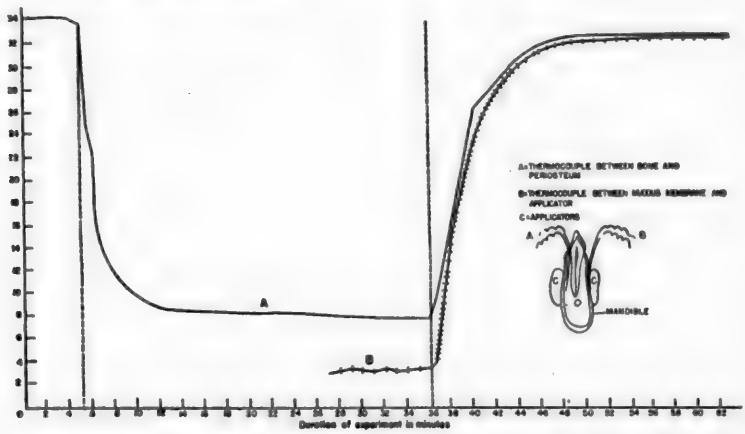

Fig. 6. Temperature measurements taken from the mandible of a dog, using copper-constantan thermocouples placed between the bone and periosteum, and mucous membrane and applicator, during local refrigeration of the jaw (temperature of refrigerant at applicators 0–1°C.)

In 4 attempts to obtain block anesthesia, i.e., chilling the mandibular nerve distal to the cavity to be treated, complete numbing of the cavity occurred in 1 instance, while in 3 only mild pain was experienced.

As reported earlier (3), no deleterious effects have been observed as following the cooling of localized areas in the maxillae or mandible. Checkups, in some cases after more than a year had elapsed, have not disclosed any gross changes in the gingivae or teeth.

Obviously, the practical use of refrigeration anesthesia in dental procedures will depend upon factors like: simplification of the applicator design, production of effective anesthesia consistently, and its usefulness in the extraction of teeth.

SUMMARY

1. Temperature measurements were taken in dogs from areas of mandibles cooled by applicators through which a refrigerant at 0° to 1°C. was circulated.

TABLE I

Cavities prepared for filling under local refrigeration

PREPARATION*	AGE**	SEX**	PAIN SENSATION TO DRILLING UNDER REFRIGERATION (1°–2° C.)	APPROXIMATE TIME TO OBTAIN EFFECTIVE ANESTHESIA	APPROXIMATE DURATION OF REFRIGERATION AT 1°–2°C.
	yrs.			*minutes*	*minutes*
30—O	20	M	None	4	10
14—M—O	20	M	None	3	12
15—O—O	20	M	None	3	7
19—DO	26	F	None	5	12
9—D	23	M	None	3½	5
10—D	23	M	Mild	4	6
4—O	19	F	Very mild	4	5
3—O	19	M	None	4	7
3—O	19	F	None	4½	6
7—D	23	M	Very mild	4	5
3—MO	19	M	None	4	10
31—D	22	F	None	5	12
15—O	19	M	None	3½	12
3—O	19	M	None	4	9
8—M	20	M	Very mild	3	8
9—M	20	M	Very mild	3	7
31—O	22	F	None	5	8
6—E	21	M	None	4	5
23—D	23	M	None	4	5
26—D	23	M	None	4	4
6—E	22	M	Mild	5	5
5—MO	19	M	None	4½	6
10—M	19	M	None	5	8
9—D	19	M	Moderately severe		17
8—D	19	M	Moderately severe		18
20—MO	19	M	None (Applicators placed between teeth 18–19)	8	12
7—D	18	M	None	5	6
31—E	20	M	Moderately severe		12
29—MO	20	M	Mild (Applicators placed between teeth 31–32)	9	14
5—DO	20	M	Mild	5	15
3—MO	24	M	None	4½	17
4—D	21	M	None	4½	8
28—DO	22	F	None	5	10
20—O	30	M	Moderate	6	18
21—E	30	M	Moderate	6	10
20—O	30	M	Mandibular nerve block (conduction anesthesia) partially effective		13
14—O—O	30	M	None	4	5
8—M	19	M	None	4	8
9—M	19	M	None	4	8
30—OI	30	M	Very mild	6	12

TABLE I—*Concluded*

PREPARATION*	AGE**	SEX**	PAIN SENSATION TO DRILLING UNDER REFRIGERATION (1°–2° C.)	APPROXIMATE TIME TO OBTAIN EFFECTIVE ANESTHESIA	APPROXIMATE DURATION OF REFRIGERATION AT 1°–2°C.
	yrs.			*minutes*	*minutes*
3—MO	24	F	None	4½	9
4—DO	24	F	None	4	5
9—D	37	M	None	4	5
19—MO	32	M	Very mild	4½	15
2—MO	24	F	None	5	12
5—DO	24	F	None	5	9
9—D	38	M	None	5½	5
3—MO	32	M	None	4½	10
30—E	32	M	Very mild (Block attempted)	10	20
30—E	32	M	None	5	20
30—MO	29	M	Severe		20
19—E	32	M	None	5	15

* O = Occlusal.
M = Mesial.
D = Distal.
E = External.
I = Internal.
** Individual subjects are grouped as to age and sex.

The lowest temperature attained in the area of the inferior alveolar nerve was 11.5°C.; between the bone and periosteum, 7.5°C.; and the lowest registered at the surface of the mucous membrane in contact with the applicator was 2.8°C.

2. Histologic investigation of nerves from canine mandibles subjected to continuous local refrigeration at 0° to 1°C. for 2-hour periods did not disclose evidence of injury to either the inferior alveolar nerves, or to the nerves of the mucous membrane and periosteum.

3. Fifty-two cavities were prepared for filling under local refrigeration at 1° to 2°C. in the teeth of 33 members of the naval service. In 34 instances (63%), complete anesthesia was obtained; in thirteen (25%), very mild to moderate pain was experienced; and in five (10%), there was little or no anesthesia.

The author is indebted to Captain A. P. Black, MC(S), USNR, for initiating this study; to Lieutenant C. R. Spealman, H(S), USNR, for assistance in the temperature studies on dogs; to Lieutenant (jg) A. M. Laskey, H(W), USNR, who prepared the nerve tissue sections; to Commander J. L. Tullis, (MC), USN, and to Lt. Commander R. H. Pudenz, MC(S), USNR, who assisted in histologic interpretation.

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THE RELATION OF PENICILLIN TO INDUCED RAT DENTAL CARIES AND ORAL L. ACIDOPHILUS[1]

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A diet consisting largely of coarse-ground corn meal will usually cause a high incidence of carious-like lesions in molar teeth of the white rat (1). Either fluoride or iodoacetate in the diet and drinking water, however, will markedly inhibit this induced form of rat caries (2). Preliminary results now indicate an inhibition of such rat caries and a striking reduction in counts of oral *L. acidophilus* by adding penicillin to rats' food and drinking water. In vitro, *L. acidophilus* is susceptible to penicillin (3). Other studies (4) have demonstrated a rapid fall in the total bacteria of the oral cavity after application of penicillin pastilles.

In this study experimental rats, matched with litter-mate controls, received a corn meal caries-inducing diet containing 75 units of penicillin[4] per gram and drank water containing 75 units per ml. Control rats of the pairs received no penicillin. The penicillin diet and penicillin drinking water were renewed daily, numerous assays (5, 6) having revealed practically no deterioration of penicillin in the diet and water after standing 24 hours in food cups and in drinking fountains which were cleaned and sterilized daily.

After 125 days on experiment the rats were sacrificed and their teeth diagnosed microscopically for caries and scored according to a standard procedure (7). As shown in Table I no caries appeared in the test (penicillin) rats whereas 50.0% of the control rats had carious teeth. Although severity and incidence of caries in the control animals was less than usual, the outcome in the control animals contrasts strikingly with the negative caries results for rats given penicillin.

To obtain the plate counts for oral *L. acidophilus* (Table II)[5] tooth surfaces were thoroughly scrubbed with a small cotton swab, and the swab placed immediately in a measured quantity (5 ml. or 2 ml.) of 1% dextrose meat infusion broth (pH 5.0). An aliquot portion (0.2 ml.) of this broth was cultured on a tomato juice agar media (pH 5.0) and the *L. acidophilus* colonies counted after 96 hours incubation (8). In 7 instances as shown in Table II, plate counts for penicillin rats were mostly negative or yielded less than 50 colonies of *L. acidophilus* organisms. The great majority of control rats on the other hand, developed upwards of 50 colonies per plate.

[1] Received for publication June 12, 1946.

[2] Senior Biochemist, Dental Research Section, Division of Physiology.

[3] Senior Assistant Surgeon, Division of Infectious Diseases.

[4] Sodium salt of Penicillin.

[5] We are indebted to Dental Surgeon F. A. Arnold, Jr. and to Senior Assistant Dental Surgeon Stanley J. Ruzicka, National Institute of Health, for assistance in making these plate counts.

Sufficient penicillin was not ingested by these rats (or it was destroyed by gastric acidity) to produce an appreciable blood level, 2 assays of blood (9) taken on successive days from 8 representative penicillin rats having yielded negative

TABLE I

Effect of Penicillin on Induced Rat Caries

Number of rats on experiment	82	82
Number of litters represented	43	43
Average number of days on experiment	118	118
Treatment	control	penicillin
Rat Caries Diagnosis		
Number of rats which were carious	41	0
Per cent of rats which were carious	50.0	—
Number of teeth which were carious	67	0
Per cent of teeth which were carious	6.8	—
Average caries score per carious rat*	5.4	—

* See Ref. 7.

TABLE II

Results of L. acidophilus Plate Counts for Control "C" and Penicillin "P" Rats

Experiment	"A"						"B"							
Count No.	1		2		3		4		5		6		7	
Dilution Factor*	5 cc.		5 cc.		2 cc.		2 cc.		2 cc.		2 cc.		2 cc.	
Rats Treatment	C	P	C	P	C	P	C	P	C	P	C	P	C	P
Total counts	26	30	27	32	27	31	21	21	21	21	21	21	21	21
Negative counts	1	25	3	27	2	24	5	13	7	14	4	14	4	20
Less than 50	12	5	14	5	11	7	3	0	3	0	4	0	3	0
50–500	8	0	10	0	14	0	3	0	4	0	1	0	8	0
500–1000	5	0	0	0	0	0	1	0	0	0	2	0	0	0
1000 and over	0	0	0	0	0	0	3	0	0	0	1	0	0	0
Plate overgrown†	0	0	0	0	0	0	6	8	7	7	9	7	6	1

* The volume of broth suspension was 5.0 cc. or 2.0 cc. The volume taken for plating was 0.2 cc., except in Count No. 7 where 0.1 cc. was plated.

† Plates overgrown with molds or spreading type colonies.

results. The inhibition of dental caries as brought about by this oral administration of penicillin, therefore, would appear to be localized within the oral cavity.

The effect of penicillin on food and water consumption and on daily gain in body weight was observed in 12 pairs of rats over a 14-week period. These results, averaged daily as follows, indicate no differences between control and penicillin rats.

	Food Intake (gm)	Water Intake (gm)	Body Gain (gm)
Control rats	12.6	17.9	2.2
Penicillin rats	13.2	19.1	2.2

Dental caries has long been identified with chemico-parasitic etiology (10), the *L. acidophilus* organism in particular being associated with the prognosis of the disease (11). There is evidence to support the chemico-parasitic theory in the discovery that iodoacetate inhibits rat caries, the iodoacetate acting presumably as an antienzymatic agent. In the same way if fluorine functions antienzymatically when inhibiting dental caries the mode of action would agree with the above caries theory. The inhibitory effect of fluoride has already been associated with the reduction of human dental caries (12).

Now that penicillin, an antibacterial agent, has been shown to inhibit induced rat caries, and to reduce the oral *L. acidophilus* counts, there seems to be additional evidence of an association of *L. acidophilus*, and perhaps other bacteria, with dental caries etiology. Accordingly, these results suggest that human dental caries may respond to penicillin therapy.

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A STUDY OF THE SYRIAN HAMSTER, ITS MOLARS, AND THEIR LESIONS[1]

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Most investigators who have studied experimental dental caries in the rat have depended principally upon a hard, coarse particle, fracture producing diet (1, 2) to induce carious lesions. The fact that the development of lesions in this type of caries is apparently dependent upon the previous occurrence of fractures has led to considerable discussion in the literature concerning the applicability of experimental results toward solution of the problems of dental caries in man. However, the discovery of Arnold (3) in 1942 that dental decay is common in the molars of Syrian hamsters fed on a laboratory diet containing a large amount of soft or finely ground carbohydrate and no coarse particles has resulted in the possibility of studying experimental caries in animals in the absence of the fracture factor.

Extensive experiments with caries-producing diets and studies of the hamster dentition had been underway in this laboratory when the preliminary publications of Keyes, Dale, and Lazansky (4, 5, 6) appeared and clearly indicated the potentialities of the hamster in dental caries research.

This report comprises part of the findings obtained in a study of the relationship of various dietary factors, the oral bacterial flora, and the incidence of dental lesions in the hamster (7) as well as some observations upon the morphology and growth of its molars and the pathological picture of their lesions.

CLASSIFICATION AND DESCRIPTION OF THE HAMSTER

The Syrian or golden hamster, *Cricetus auratus*, has been the object of some diversity of opinion among naturalists as to its exact taxonomic status,[2] but at present the animal is placed in the subfamily *Cricetinae*.[3] Argyropulo (8),

[1] Presented at a meeting of the Chicago Section of the International Association for Dental Research, October 12, 1945. Received for publication August 22, 1946.

[2] The hamster's phylogenetic relationship:

Order *Rodentia*
Family *Muridae*
Subfamily *Cricetinae*
Genus *Cricetus*
Subgenus *Mesocricetus*
Species *auratus*

[3] The common laboratory animals, rats (*Rattus*) and mice (*Mus*) belong to the same family (*Muridae*) as the hamster, but are placed in another subfamily (*Murinae*), primarily on the basis of the anatomy of the molar teeth.

making a comprehensive survey of all hamsters, placed the species *auratus* in the subgenus Mesocricetus on the basis of mandibular osseous structures and fur color distribution. The name *auratus* has reference to the golden-brown dorsal fur of the species which has its natural geographical distribution in parts of Syria, Persia, and Turkey.

Characterized by extensive bilateral cheek pouches, a stubby tail, soft fur which is golden-brown dorsally and grayish-white ventrally with a darker band across the chest, a normal adult hamster weighs an average 100 gm. and when standing quandrupedally is approximately 10 cm. long and 5 cm. tall. The skull, as is characteristic of all rodents, possesses prominently developed osseous

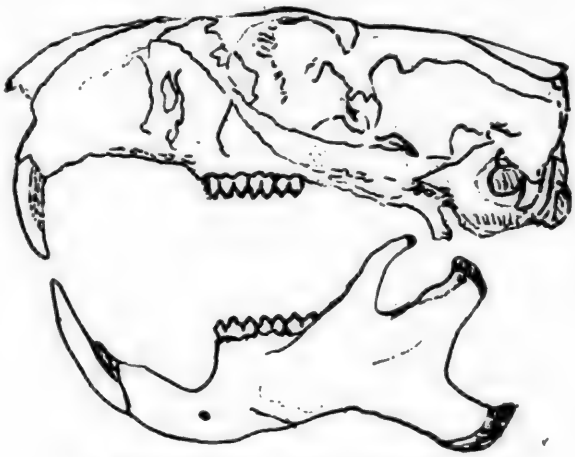

Fig. 1. Sketch of an adult hamster skull. (2½ ×)

structures supporting the powerful and sharp incisor teeth. Separated from the 4 incisors by wide diastemata, there are 12 molars. Canines are absent as in all the *Rodentia* and, as in most members of this order (excluding the duplicidentates which are now usually placed in the order *Lagamorpha*), there are no premolar teeth (*fig. 1*).

The dental formula for the hamster is:

$$\mathrm{I}\,\frac{1}{1}\,\mathrm{C}\,\frac{0}{0}\,\mathrm{PM}\,\frac{0}{0}\,\mathrm{M}\,\frac{3}{3}$$

When the mandible is at rest in retrusion, there is bilateral occlusion of the molar teeth. The hamster's dentition is considered to be heterodont (having teeth not all alike) and monophyodont (developing but one set of teeth). The molars have short (brachyodont) and multituberculate (polybunodont) crowns and well developed roots.

Molar Teeth

Morphology.—Unlike its continuously-growing and yellow-pigmented incisor teeth, the hamster's molars, which are limited in growth, are not pigmented but

appear white or slightly pink in vivo. Enamel completely covers the molar crowns in contra-distinction to the enamel-bare cusps of the rat molar.

The fundamental pattern of all hamster molars is characterized by 3 pairs of cusps which are arranged on a longitudinal antero-posterior axis. This pattern is gradually modified from the first through the second and third molars. The paracusps,[4] usually paired, are the smallest cusps on the crown and are located farthest mesially; in the second and third molars they are very much reduced. The paired mesocusps, meso-centrally located, are prominent in all the molars, and the paired distocusps, distally located on the crown, are equally prominent. Separating the 2 cusps of each pair is a rather deep occlusal fossa or occlusal groove and between each pair of cusps are wide triangular buccolingual sulci, respectively sub-occlusal and supra-occlusal in the mandibular and maxillary molars.

Calcification and Eruption.—In order to know how long the molar teeth had been exposed to the oral environment of the diet and the bacterial flora, it became necessary to investigate the eruption time of each molar. Likewise, to know what effect the diet may have in tooth formation, it became necessary to learn the time of calcification of each molar. To study the calcification and eruption of these teeth, the skull of the sacrificed animal was cut longitudinally into halves with a dental separating disk. One half was studied under magnification of 15 and 20 diameters, most of the soft tissue was removed, and subsequently the hemi-skull was roentgenographed; the other half was placed in 10% neutral formalin for future sectioning (*fig. 2*). The tabulated data obtained by such observations, appearing in Table I, closely corroborate and extend the finding of Keyes and Dale (4). There were slight variations in calcification and eruption, especially for the third molar, which may be related to the rate of animal growth and litter size. Sex apparently was not a factor in these variations although the adult female often is larger in body size than the male. There may also be systemic dietary influences on the rates of calcification and eruption, but the effects of these factors were not studied at this time.

Molar Lesions

Description.—The carious lesions induced in the molars of the hamster may occur in a variety of areas. There is evidence, however, that they usually begin in certain limited, susceptible locations. As has also been pointed out by other investigators, the initial lesions are of 2 general types: (A) Those found in the grooves and fossae as well as in the sulci, and (B) those found on smooth cervical or proximal surfaces.

Grossly, the occlusal as well as the sub- and supra-occlusal surface lesions encountered in this study began as small linear breaks in the enamel and progressively enlarged until there was virtually complete destruction of the crown with pulp exposure (*fig. 3*). Lateral cervical lesions commenced on the smooth surface just below the cervico-enamel ridge with the development of an opaque white area but without loss of surface continuity. Subsequently, a break in the

[4] To maintain uniformity of description regarding the anatomy of the molars, the nomenclature recently used by Keyes and Dale (4) is here also employed with minor modification.

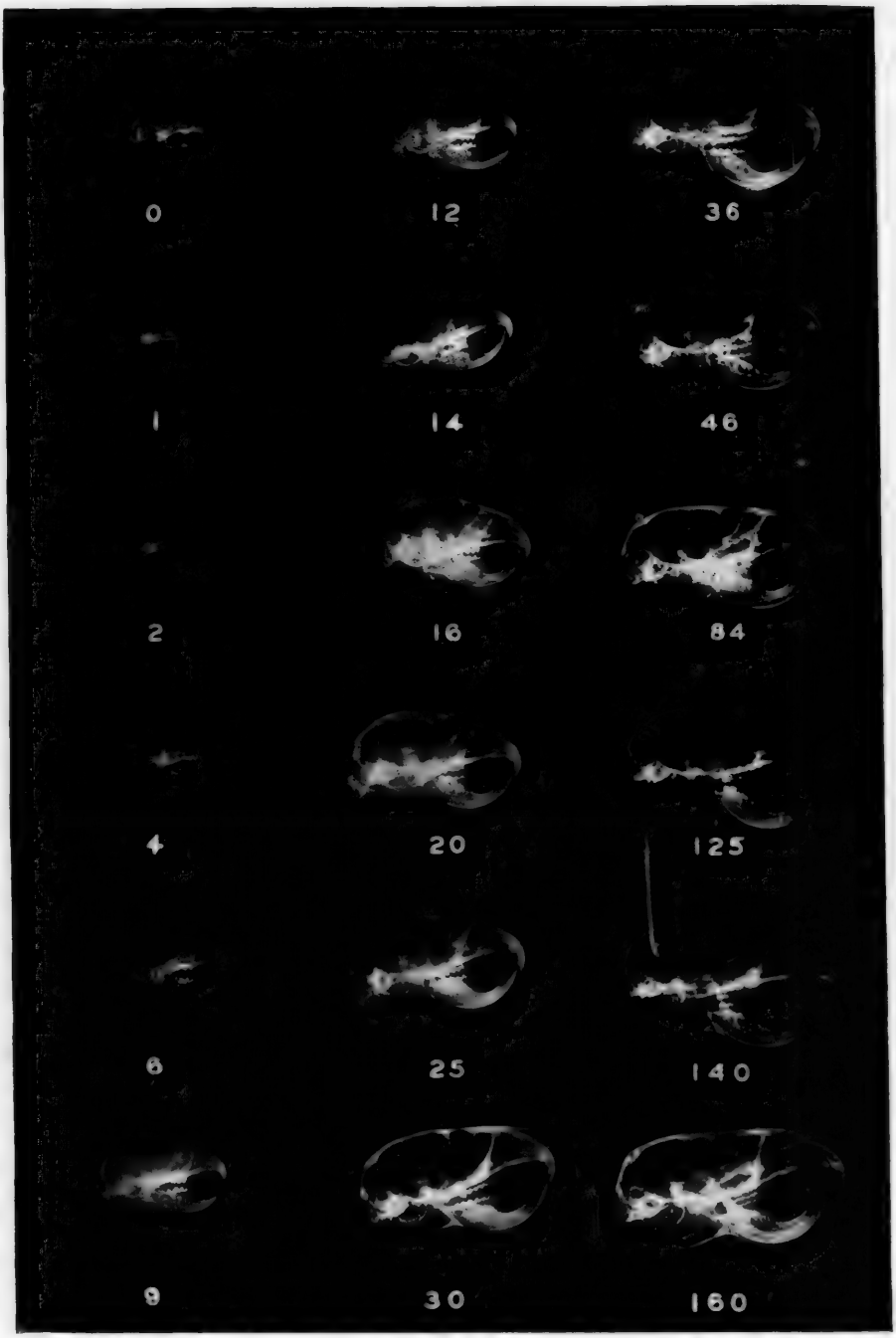

Fig. 2. Cephalo-roentgenographs of a series of hamsters at different ages showing calcification and eruption of the molar teeth as well as growth of the skull. The numbers indicate postnatal age of the animal in days. (1×)

enamel surface developed and cavitation progressed, causing cusps to fracture and accelerating the final destruction of the whole crown (*fig. 4*). The gingivae, which normally cover much of the lateral surface of the molars, were never

TABLE I

Calcification and eruption of hamster molar teeth as observed directly or roentgenographically
Figures are expressed in days post-natally

HAMSTER MOLARS	OSSEOUS CRYPT FIRST VISIBLE	FIRST EVIDENCE OF CALCIFICATION	CROWN COMPLETELY CALCIFIED	FIRST EVIDENCE OF ERUPTION INTO THE ORAL CAVITY	COMPLETE ERUPTION; TEETH IN OCCLUSION	ROOTS COMPLETELY CALCIFIED
Maxillary 1st molar.....	at birth	2	8	9	8–9	35–40
Mandibular 1st molar...	at birth	2	8	8		25–30
Maxillary 2nd molar	2	6	13	14	17–18	42–46
Mandibular 2nd molar ..	2	5	12	12		36–38
Maxillary 3rd molar ...	12	20–22	30–34	32–34	42–46	90–100
Mandibular 3rd molar .	12	18	25–28	29–30		75–85

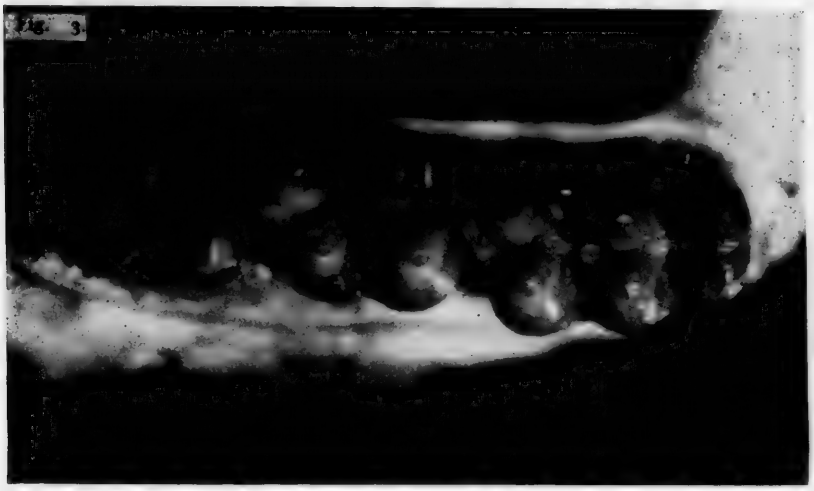

Fig. 3. Occlusal view of right, maxillary quadrant depicting moderately sized occlusal cavity in third molar without cusp fracture.

found over these surfaces when there was a cavity present. Interproximal surface lesions, which were difficult to observe, were seen less frequently. They apparently begin, as in the interproximal lesions of human teeth, at a point gingival to but near the contact point, although in the case of the hamster there is a rather large contact surface which extends to the level of the marginal ridge.

In molars which were slightly mal-aligned as to their approximating surfaces, such interproximal lesions occurred more often. Cavitation progressed in these lesions also and eventually extended to involve multiple adjacent surfaces. Microscopically, decalcified sections of extensive molar lesions showed vast destruction of hard tissues and pulp exposure with apical progression of degeneration and necrosis, but usually without periapical tissue destruction as in human succedaneous molars in a similar state of carious involvement (*fig. 5*).

All these observations indicate that hamster molar lesions, in their appearance, site, and progress, bear a resemblance to the type of dental caries found in the premolars and molars of man.

Fig. 4. Smooth surface lesions on the buccal aspect of mandibular molars. First molar has extensive cervical lesion which circumvents the mesial portion of the tooth. Earlier stage of this type of lesion would show only a white opaqueness denoting decalcification, whereas a much later stage would show a fracture of the undermined paracusp. The third molar has a smaller cavity with characteristic white decalcified areas nearby. (18×)

Recording of Lesions.—A method of recording the site, size and number of carious lesions and a system of charting, similar to that devised by Bodecker (9, 10) for study of human dentition and that used by Cox, Dodds, Dixon and Matuschak (11) for molar dentition of the rat, which were used in the earlier part of the work were discarded in favor of the charting and scoring procedures used in study of the hamster by Keyes. Keyes' methods, with minor modifications, were used throughout the study and earlier data were rescored on this basis. The maximum total score for any one molar crown by these methods of evaluation was equal to the sum of all its unit surfaces multiplied by 3, since that was the maximum degree of cavitation. Thus a score value of 282 would indicate complete destruction of all 12 molars. Hence, the caries index for an animal on this basis, would be that percentage of the whole molar dentition with the value 282, which was destroyed by dental caries.

Distribution.—In a study of the oral bacterial flora of the hamster, the detailed

results of which appear in another report, the molar lesions in 179 animals on various diets were observed and recorded. On the basis of the over-all caries

Fig. 5. Microscopic decalcified section through the first and second mandibular molars. (32×) Second molar has very extensive dentinal lesion with much destruction of tooth substance resulting in pulp exposure, degeneration, and necrosis. First molar has no large lesion and had normal enamel (removed in decalcifying process) covering the cusps. Dentin and pulp is also normal.

TABLE II

Distribution of molar lesions

CARIES INDEX	NUMBER OF HAMSTERS	AVERAGE NUMBER PER ANIMAL				
		Molars affected	Lesions			Pulp exposures
			Smooth surfaces	Occlusal surfaces	Total	
20 –29.9	11	11.1	11.0	5.9	16.9	5.6
10 –19.9	12	10.2	8.8	5.1	13.9	3.3
5 – 9.9	26	8.6	6.8	4.6	11.4	1.9
1 – 4.9	73	4.0	2.6	2.4	5.0	0.3
0.1– 0.9	36	1.7	0.86	0.88	1.8	0
0	19	0	0	0	0	0

index, the distributions of the number of molar teeth involved, the types of lesions, and the number of pulp exposures are charted in Table II.

Nineteen hamsters were found entirely caries-free. Those animals with a low caries index had an almost equal distribution of lesions in the fossae and sulci of the occlusal surfaces and in the smooth surfaces, whereas those with a higher caries index had a predominence of the smooth surface lesions. In 2 hamsters (not recorded in Table II) with the unusually high caries index of 40.8 and 45.4, the lesions involved the pulps and were so extensive that it was difficult to ascertain whether they began in the fissured occlusal surfaces or on the smooth surfaces of the teeth.

Of the animals studied, there was only a slightly greater caries index for the males as compared to the females,[5] although equal proportions of males and females were caries-free. There seemed to be no relationship among the caries indices of littermates on the same diets. However, a 20% greater caries index was found in maxillary molars as compared to mandibular molars, whereas no significant difference occurred between the right and left molar teeth of the animals. The third molars were involved most frequently and the maxillary third molars had a caries score almost twice that of the corresponding mandibular third molars. The mandibular first molars were next in degree of caries involvement, having a caries score somewhat less than half that of the mandibular third molars.

It should be borne in mind that the animals reported in this study were not all subjected to the same dietary regimens. There were 17 diet groups. The basal ration was the same for most of the groups, the diets differing by modification of the vitamin components. In this general, over-all summary of the types and distribution of lesions no account has been taken of the possible differences with respect to the imposed dietary factors.

SUMMARY

The Syrian hamster, *Cricetus auratus*, is described as to its phylogenetic relationship and anatomical characteristics pertaining to the dentition. The calcification, eruption, and morphology of the molar dentition is described and discussed and the distribution and types of carious lesions in the molar teeth are also described.

The author is grateful to Dr. R. W. Harrison and Dr. J. R. Blayney for helpful suggestions and advice.

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THE ORAL BACTERIAL FLORA AS RELATED TO DENTAL CARIES IN THE SYRIAN HAMSTER[1]

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It has been demonstrated by Arnold (1), by Dale, Lazansky and Keyes (2), and by more recent observations (3) in this laboratory that the Syrian hamster, *Cricetus auratus*, when maintained on various kinds of soft diets will develop lesions in the molar teeth which closely resemble dental caries in man. It seems apparent, as has been demonstrated many times with other species, that bacteria must have an important role in relation to the effects of caries-producing foods on the teeth of the hamster. Since there were no reports in the literature on the subject, this study was undertaken as a preliminary investigation of the oral bacterial flora of the hamster.

Although it has been recognized for many years that acid production in the mouth is an accompaniment of dental decay and although the theories of W. D. Miller (4), pointing to the acid producing bacteria of the mouth as the cause of dental caries, have been generally accepted, the accumulation of detailed experimental evidence has been slow. For example, not until comparatively recently was it demonstrated, by Stephan (5), that there is rapid acid production in the immediate vicinity of the tooth surface when carbohydrate is available to the microorganisms concentrated in the adherent plaque.[2] He has shown that there is a rapid, sharp increase in acidity on the tooth surfaces usually within a minute or 2 after the mouth is rinsed with a glucose solution. This drop in pH was greatest and lasted longest in caries active cases, although it averaged pH 5.2 for the different caries activity groups studied by Stephan.

For the purposes of the present study it has been assumed that microorganisms which may be related to caries activity are aciduric in nature; that the microorganisms concerned in the process must be acid tolerant in order to survive the degree of acidity which occurs on the surface of a tooth where decalcification is taking place. Consequently, the microorganisms investigated have been limited primarily to those which grew on acid culture media.

The hamsters were obtained from a colony used in experimental diet studies which were being conducted in this laboratory at the time these investigations were being made. Each of the animals used for bacteriological study had been

[1] Presented at a meeting of the Chicago Section of the International Association for Dental Research, March 8, 1946. Received for publication August 22, 1946.

[2] The bacterial plaque has been described (6) as a colorless, thin, tenacious, felt-like mass of material, closely adherent to the tooth surface and containing filamentous bacteria which enmesh other bacterial forms.

fed on one of several experimental diets for a period of 100 days, having been placed on these diets at the age of 25 to 30 days.

Preliminary observations were made with cultures obtained by swabbing the surfaces of the molar teeth. Neither the numbers nor the kinds of microorganisms isolated from these samples varied to any significant degree in relation to the differences between the several diets ingested by the various animals. It seemed reasonable to expect, however, that some detectable quantitative relationship might exist between the number and/or variety of the oral microorganisms and the presence or absence of various dietary components, or if none should be found, that at least it would be possible to detect modifications of the flora associated with differences in caries producing effects of the several diets. Consequently, an attempt to conduct a more quantitative study was made.

MATERIALS AND METHODS

The majority of the hamsters studied had received a vitamin B-free basic diet,[3] fortified with alpha tocopherol, vitamin K, and a commercial preparation[4] containing vitamins A and D, and supplemented with mixtures of varying components of the vitamin B complex: riboflavin, calcium pantothenate, thiamine, pyridoxine, nicotinic acid, inositol, para-amino-benzoic acid, choline, and biotin. These animals were divided into 14 dietary groups. The food for each group was lacking in one of the fortifying or supplementing vitamin components, with the exception of the groups on Diets I, VIII, and XIV which were complete. Whenever one of the components was deleted from the diet, it was supplied by intraperitoneal injection in amounts reported in the literature to be nutritionally adequate. The vitamin deficiencies in the diets ingested by the animals used in this study were as follows: Diet II—riboflavin, Diet III—calcium pantothenate, Diet IV—thiamine, Diet V—pyridoxine, Diet VI—nicotinic acid, Diet VII—inositol, Diet IX—para-amino-benzoic acid, Diet XI—choline, Diet XII—biotin, and Diet XIII—alpha tocopherol. The complete diet, i.e., Diet XIV, was the same as Diet I except that dextrin was substituted for sucrose; Diet VIII differed in its salt mixture from Diet I.

It must be pointed out that the present study is concerned, first, with the local effects rather than with the effects on the general, systemic nutritional status of the animal. Second, it is concerned with the composition of the food adherent to the teeth and perhaps otherwise trapped in the hamster's mouth and third, with the comparison of the effects the various diets produced upon the oral flora.

In addition to the animals from the diet groups listed, a few were from a group of hamsters which had been on the basic diet with the addition of all the vitamins except riboflavin and vitamin K in place of which was substituted a commercial preparation[5] rich in carotene, riboflavin, and vitamin K. Three other groups of

[3] Vitamin B test diet from S.M.A. Corp. Diet contains hydrogenated vegetable oil, sucrose, vitamin-free casein, salt mixture, and sodium chloride.

[4] "Vi-delta" contains vitamin A and D (Viosterol) prepared by the Lederle Laboratories, Inc., New York, N. Y.

[5] "Cerophyl"; contains vitamins A, C, G, and K, as well as chlorophyl; prepared by the Cerophyl Laboratories, Inc., Kansas City, Mo.

animals were similarly studied. These were fed on 3 different commercial preparations[6,7,8] which were complete in all the essential dietary components but contained some fluorine.[9] The water, which all animals received *ad libitum*, contained no fluorine.

At the termination of the 100 day feeding period, each animal was placed in a closed jar and anesthetized by inhalation of vaporized ethyl ether. A loop of 18 gauge wire, attached to a ringstand, was flamed, allowed to cool and then used to suspend the anesthetized hamster by its 2 prominent maxillary incisor teeth. The mandible was retracted with a small sterile wooden applicator and a concentrated light source was used to provide illumination of the oral cavity. A specially prepared sterile glass tube was used to aspirate saliva and material adherent to the molar teeth. The tube consisted of 6 mm. glass tubing about 15 cm. in length with one end curved and drawn to a fine opening. This end was provided with a small, circumventing piece of rubber with which the molar teeth were massaged to assist in the removal of adherent particles. The other end of the aspirating tube was attached by rubber tubing to a small vacuum pump which produced a negative pressure of 5 cm. in a standard mercury manometer. The bore of the aspirating tube proved to be large enough and the suction pressure low enough to prevent loss of fluid by movement of bubbles through the aspirator. In the minute or 2 during which the hamster was in the third stage of anesthesia, about 0.02 to 0.03 cc. of saliva and tooth-adherent debris could be aspirated from the perimolar regions. The collected specimen was expelled from the glass aspirator into a serological tube and, after air bubbles had been eliminated, 0.01 cc. was accurately withdrawn with a serological pipette and transferred to a Wassermann tube containing 2 cc. of enriched acid broth.[10] After being thoroughly mixed, 0.2 cc. was removed from this tube and spread by gravity flow over the surface of enriched acid agar.[11] Acid agar, poured plates were used

[6] "Rockland Rat Diet" prepared by Arcady Farms Milling Co., Chicago, Ill.

[7] "Purina Dog Chow Checkers" prepared by Ralston Purina Co., St. Louis, Mo.

[8] A special "Purina" diet with a low fluorine content was prepared for this study by Ralston Purina Co.

[9] According to assays, kindly made for us under the direction of Dr. D. A. Greenwood, Department of Pharmacology, University of Chicago, sample of the "Rockland" diets averaged 17 p.p.m. F, samples of the "Purina" diet, 37.9 p.p.m., and of the special "Purina" diet, less than 0.5 p.p.m. F.

[10] The acid broth, a modification of a medium described by Weiss and Rettger (7), was prepared by the following formula:

"Bacto-Difco" peptone	5 gm.
"Difco"-peptonized milk	10 gm.
"Difco"-yeast extract	5 gm.
Filtered tomato juice (as for Kulp's media)	400 cc.
Distilled water	600 cc.

These ingredients are heated, filtered if necessary, and adjusted to pH 5.3 so the final reaction after autoclaving will be pH 5.0–5.2. The medium is then tubed and autoclaved.

[11] The acid agar was prepared by the same formula as the broth, but with the addition of 2½% agar. However, the peptone, milk, yeast, water, and agar is first autoclaved, cooled to 50°C. and then the filtered tomato juice is added. The pH adjustment is made, final autoclaving is done, and the plates are poured with the medium at 50° C.

in parallel with the surface inoculated plates in several tests but since cultures made in this manner produced surface and subsurface colonies comparable in number and kind to the surface colonies obtained in the routine procedure, the use of the poured plate was discontinued.

For study of non-aciduric bacteria, cultures were grown on veal infusion agar and blood agar.

Four days' incubation at 37.5°C., anaerobically,[12] was sufficient to reach the peak of bacterial growth. The colonies were then classified on the basis of colonial and cellular morphology, counted, and subcultured for further study. The colony count of each plate was recorded as the number of viable aciduric bacteria per cubic millimeter of specimen. The foregoing procedure was developed as an attempt at quantitative sampling of the perimolar flora and was used routinely on all the hamsters studied.

In a number of instances the buccal pouches of the hamster were also sampled for their bacterial flora. The empty pouch of the anesthetized animal was swabbed with a sterile cotton applicator with which a tube of broth was inoculated.

Bacterial specimens were also taken from the carious debris of the molar lesions soon after the animals were sacrificed. The teeth were wiped with alcohol, a fine, sterile broach was forced into the softened debris of the lesion, and the broach tip was then cultured.

Fecal cultures were made from several of the hamsters to determine whether any of the oral forms occur in the intestine. However, the bacterial findings were so varied, even when successive fecal specimens were cultured, that the fecal samplings are considered more qualitative than quantitative.

BACTERIAL FINDINGS

Microorganisms Obtained in Culture from Tooth Surfaces

Colonies on a total of 227 acid agar plates, inoculated with perimolar specimens from 179 hamsters which had been maintained on the various diets, were examined with a binocular microscope under magnification of 7, 15, or 20 diameters.

A majority of the colonies consisted of gram positive bacilli but there were considerable numbers of filamentous forms and a moderate number of gram positive cocci including short-chained streptococci, diplococci, and a few varieties of micrococci. Only a small number of yeast colonies were encountered. All these different bacteria could be grouped into 8 categories, primarily on the basis of morphology aided by fermentation tests and immunological reactions of some of the strains:

1. Gram positive, short, coccoid bacilli, frequently in chains, occasionally slightly curved, were obtained from 2 kinds of colonies. The more common type of colony, 0.5 to 1.0 mm. in diameter, was yellowish-white, translucent, smooth and glistening, and had

[12] Although most of the aciduric oral bacteria are considered to be aerobic and facultatively anaerobic, agar plates incubated aerobically seemed to develop smaller numbers, fewer varieties, and smaller sized colonies than concurrent plates incubated anaerobically.

either a low or high dome-shaped contour. The other colony type was larger, 1.0 to 2.0 mm. in diameter, with a raised yellow center and a wide, white periphery. This colony was moderately convex with a slightly granular surface and a smooth edge. This group of bacteria resembled the Type I lactobacilli of Hadley (8).

Fermentation tests with these coccoid bacilli resulted in acid production, but no gas formation, from xylose, glucose, fructose, galactose, sucrose, maltose, and raffinose. Some cultures did not ferment lactose, nor produce acid or cause reduction in litmus milk, and none fermented dextrin, mannitol, or sorbitol (Table I).

Precipitation tests were performed with crude carbohydrate extracts of 3 different cultures of this group. These tests were made with rabbit antisera, which had been prepared in this laboratory against oral lactobacilli of human origin, and they were performed as described in previous publications (9). In the case of 2 of these 3 carbohydrate extracts, positive precipitation tests resulted with 2 different antisera. It is interesting to note that the cultures from which these 2 extracts were made fermented lactose and caused some reduction of indicator and acid formation in litmus milk, whereas the culture from which the third extract was made produced no acid in lactose and no detectable change in litmus milk.

2. Gram positive, medium sized bacilli, usually not in chains, and frequently palisaded in large aggregates, were observed in smears from a great variety of aciduric colonies. There were 8 colony forms differing mainly in minor details. They were 1.0 to 2.0 mm. in diameter, yellowish, translucent or opaque, with a white mottled center and yellow periphery. They were all moderately convex, some umbonate with edges slightly fimbriated or entire. All their surfaces were smooth, though some were dull and others were glistening in reflected light. These bacilli bear a close resemblance to Hadley's Type II lactobacilli.

Fermentation reactions showed rather consistent acid production in xylose, glucose, fructose, sucrose, maltose, and raffinose. Most cultures tested fermented galactose and lactose, but many of them did not ferment dextrin, mannitol, sorbitol, nor produce detectable reactions in litmus milk. However, there were some which showed marked glycolytic activity in mannitol and sorbitol, and caused litmus reduction and acid formation with coagulation in milk.

The crude carbohydrate extracts of 2 representative cultures were tested with 12 type-specific antisera from the laboratory stock of human oral lactobacillus antisera. One showed a positive precipitation reaction.

3. Gram positive bacilli slightly larger and longer than those just described were observed infrequently. These developed 2 different colony forms. One was yellow-gray, about 1 mm. in diameter and slightly raised with a central crater in some cases. The other colony was smaller and had a white umbonate center, though the rest of the colony was gray and flat with a lacerate margin. The cells from this colony type were different from the other in that they were slightly longer, more slender, and did not exhibit palisade formation.

4. Filamentous forms, long, curving, and of medium width, staining with both gentian violet and the counterstain, were encountered infrequently. These forms produced small, flat colonies of 0.3–0.5 mm. in diameter with glistening surfaces and entire margins. The colonies were translucent and apparently took on the yellowish color of the medium. These microorganisms were morphologically like various intermediate forms of lactobacilli.

5. A pleomorphic filamentous form was isolated in moderate numbers. Smears showed some pleomorphism consisting of a mixture of long intertwining strands and shorter, tortuous, segmented, filaments, both of which stained irregularly by Gram's method. One colony type averaged 0.5 mm. in diameter, whitish-gray in color, with a rough, flat, lace-like surface. Another was 1.0 mm. in diameter, opaque, yellow, low in convexity, but had a very rough surface and filamentous edge. A third kind, also 1.0 mm. in diameter, was rather flat, gray, and coarsely granular with a lacerate margin. These bacteria probably are rough variants of lactobacilli.

6. A series of bizarre rod-like forms were also observed. Most of them were gram variable, staining with both gentian violet and safranin; they appear only partially stained with

methylene blue. Their colonial morphology was just as varied. No attempt at identification of these microorganisms was made since they were found in only small numbers.

7. Gram positive streptococci, occurring singly, in pairs, and in short chains, were encountered frequently and usually in large numbers. The colonies were punctiform, measuring 0.25 mm. in diameter, white, translucent, slightly convex with a smooth and glistening surface and had a watery consistency. Since these cocci appeared to be morphologically and culturally identical with stock cultures of aciduric, alpha and gamma streptococci, it seems plausible to consider them as such.

8. Micrococci were encountered frequently but were not numerous. Large colonies typical of *Staphylococcus albus* were easily identified but were found only occasionally. There were many smaller colonies averaging less than 0.5 mm. in diameter, slightly yellow, translu-

TABLE I

Fermentation and immunological reactions of the most frequently found aciduric bacilli

GROUPS OF ACIDURIC BACILLI	NUMBER OF STRAINS TESTED	FERMENTATION TESTS*													IMMUNOLOGICAL TESTS**
		Xylose	Glucose	Fructose	Galactose	Lactose	Sucrose	Maltose	Raffinose	Dextrin	Mannitol	Sorbitol	Litmus milk: Acid formed	Litmus milk: Coagulation	Litmus milk: Reduction	
Group 1 (Type I lactobacilli)	7	+	+	+	+	±	+	+	+	−	−	−	±	−	+	2 out of 3 cultures showed + pp't. test
Group 2 (Type II lactobacilli)	13	+	+	+	±	±	+	+	+	−	±	±	±	±	±	1 out of 2 cultures showed + pp't. test
Groups 4 & 5 (intermediate & rough lactobacilli)	4	−	±	±	±	−	−	±	±	−	−	−	±	±	±	None tested

* + indicates acid formation; ± reaction variable; − no change. No gas was formed.

** Precipitation tests were performed with crude carbohydrate extracts of bacterial cultures and rabbit antisera prepared against oral lactobacilli of human origin.

cent, somewhat convex and glistening and with undulate edges, the cells of which stained like typical staphylococci. *Micrococcus tetragenus* was not found on the acid medium but some sarcinae were occasionally found.

It should be pointed out that in making the differential colony counts there were on occasions bacterial cells with borderline morphologies. This was disconcerting in the case of some of the aciduric streptococci and certain of the group of short coccoid lactobacilli. There was also some difficulty encountered in differentiating some of the longer coccoid lactobacilli from the medium sized lactobacilli. Subcultures and identifying tests were made in many instances to clarify these cases but it was not feasible to do so in every case.

Cultures from Other Oral Areas

Buccal pouches.—The voluminous buccal pouches of the hamster, which are ordinarily used for food transport and storage, were also sampled for their

bacterial flora. Since there are no salivary ducts directly connected with these pouches, the mucous membrane is relatively dry. Cultures were obtained by swabbing the empty pouches with sterile cotton applicators which were then used to inoculate tubes of acid broth. These were incubated and examined for microorganisms.

In the cases sampled, there was a great predominance of gram positive, medium sized rod forms, both chained and palisaded, identical with the medium sized lactobacilli found in the molar regions. Only a few cocci, gram positive diplococci, were observed.

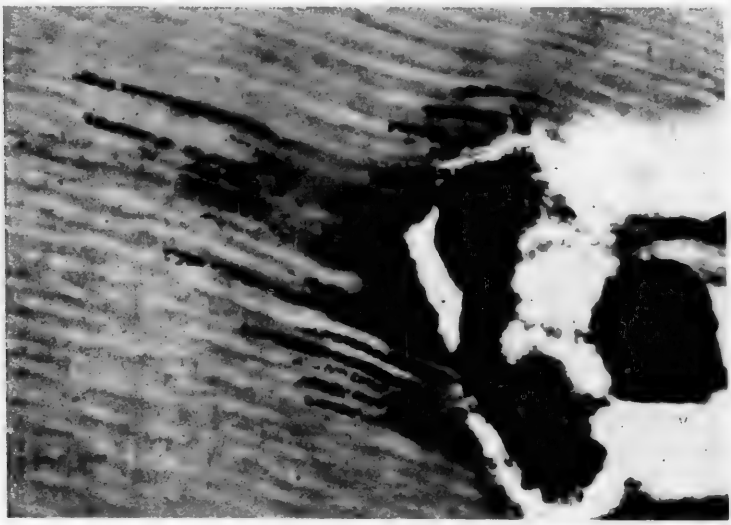

Fig. 1. Photomicrograph of an initial carious lesion in the dentin of a hamster molar depicting invasion of the dentinal tubules by bacteria. (Brown and Brenn stain × 1300)

Carious cavities.—It can readily be demonstrated by microscopic examination that even in very early lesions of the hamster molars, bacteria seem to invade the dentinal tubules (*fig. 1*) before mass destruction of the tissue takes place.

Hence a number of molar teeth with early and also with more extensive lesions in their surfaces were sampled for bacterial growth. After swabbing the molars of the sacrificed animal with 70% alcohol, a sterile, fine broach was forced into the softened dentin; the broach tip was then cultured in acid broth. In most cases the predominating microorganisms were slender, long and short, gram positive lactobacilli occurring in chains and palisades. Cultures made from cavities in which the dentin was hard yielded no growth. Cultures from cavities with softened dentin contained numerous short lactobacilli. Specimens from deep lesions exposing the pulp showed growth of cocci and a few short and long lactobacilli in the acid broth. Veal infusion broth cultures contained only a few gram positive bacilli, but a predominant quantity of gram positive, short-chained streptococci. This observation may indicate a change in the predominating

flora from the more aciduric forms to the less acid-resisting bacteria as one approaches the pulp.

Microorganisms cultured from the fecal flora.—It was also of interest to investigate the fecal aciduric flora and to make qualitative comparisons with that of the perimolar region, to learn if there might be any indication of a correlation such as was found in a study of children by Harrison and Opal (10).

Medium sized lactobacilli were the predominant organisms in cultures from almost every fecal specimen. Colonies composed of intertwined filamentous forms also occurred in rather large numbers. Only a very few colonies of streptococci were found, whereas a number of typical *Staphylococcus albus* colonies were encountered. A large proportion of large white and smaller tan colored colonies of yeasts were found, in contrast to the few observed in the oral specimens.

Non-aciduric Flora of the Molar Teeth Surfaces

The flora of the perimolar areas that would grow on media of neutral pH was investigated to a limited degree. Veal infusion agar plates and blood agar plates were inoculated concurrently with the acid plates, equal amounts of inoculum being used for each plate.

Microorganisms of a wide variety were found to grow under both aerobic and anaerobic conditions. On a number of plates a spreading form of *Proteus* overgrew the entire surface. To prevent the spread of this motile bacterium, agar plates were prepared to contain 6% by volume of 95% ethanol according to the method of Floyd and Dack (11). In addition to lactobacilli, which grew rather infrequently and poorly on this medium, there were *Staphylococcus albus* and various streptococci.

Relationship of Oral Aciduric Bacteria to the Diets and to the Caries Indices

Table II shows the relationship of the bacterial findings and the various diets which may have had an influence upon the oral flora. Also included for comparative purposes are the caries indices of the animals studied bacteriologically as well as those of the total number or hamsters maintained on each diet. Thus the average aciduric bacterial counts refer only to the smaller groups of hamsters whose perimolar regions were cultured and not to the total number of animals whose average caries indices may differ rather markedly in some instances from those of the animals studied.

From the table it can be seen that animals on Diet XI where choline was absent from the oral environment and where many extensive molar lesions developed, the total bacterial count as well as the lactobacillus count was high. The lactobacillus count was also high in Diet V where pyridoxine was not present and in Diet VIII where no factor was absent from the oral environment. On the other hand, when para-amino-benzoic acid was deleted from the oral environment, the aciduric streptococcus count and the caries index were both high, although the caries index for the total group on this diet was much lower.

By contrast, very few aciduric bacteria were isolated from animals having a low caries index and receiving a commercial diet containing 37.9 p.p.m. fluorine. However, animals on two other commercial diets with fluorine contents of 17 and

TABLE II

Relationship of diets and the oral aciduric bacteria cultured

DIETS	TOTAL NUMBER OF ANIMALS STUDIED			ANIMALS STUDIED BACTERIOLOGICALLY					
	No.	Caries index		No.	Average caries index	Average bacterial colony count per mm³ of perimolar specimen			
		Average	Range			Total	Lactobacilli	Streptococci	Staphylococci
I; contains all factors[1]	56	5.0	0 –45.4	18	7.2	938	536	271	113
II; lacks riboflavin	39	3.5	0 – 8.7	4	4.9	588	554	0	33
III; lacks Ca pantothenate	30	3.9	0.6–14.	14	2.0	464	125	98	223
IV; lacks thiamine	31	5.5	0.1–24.7	12	1.9	577	461	35	90
V; lacks pyridoxine	24	7.1	0.6–12.9	7	11.4	1,470	1,070	392	27
VI; lacks nicotinic acid	34	3.2	0 –13.6	8	6.4	673	296	258	120
VII; lacks inositol	43	4.3	0 –13.0	12	7.1	711	485	179	44
VIII; contains all factors[2]	49	6.8	0 –56.5	9	8.3	1,302	1,301	0	0
IX; lacks para-amino benzoic acid	67	4.6	0 –21.3	7	12.9	2,117	694	1,426	0
XI; lacks choline	49	10.2	0 –26.7	8	21.5	1,666	1,578	89	0
XII; lacks biotin	19	3.6	0 – 8.8	8	4.3	798	303	333	0
XIII; lacks alpha tocopherol	45	3.0	0.5– 8.5	8	1.8	293	268	25	0
XIV; same as Diet I[3]	29	3.1	0 –12.5	6	3.4	307	307	0	0
Enriched[4] with A, C, G, K	9	3.5	1.1– 6.0	9	3.5	265	153	45	29
"Rockland Rat Diet," 17 p.p.m. F	13	0.7	0 – 2.8	13	0.7	733	530	168	31
"Purina Dog Chow," 37.9 p.p.m. F	20	1.0	0 – 4.6	20	1.0	181	107	19	18
Special "Purina Diet," 0.5 p.p.m. F	35	0.6	0 – 9.7	16	0.1	723	692	27	0

[1] Vitamins A, D, E, K, and thiamin, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, para-amino-benzoic acid, choline, and biotin.

[2] Same as Diet I, except that the salt mixture in the basic diet differed.

[3] A complete diet except that dextrin was substituted for sucrose.

[4] Basic diet enriched by adding "Cerophyl."

TABLE III

Dental caries and oral aciduric bacteria in the hamster

CARIES INDEX	NO. OF ANIMALS	AVERAGE NUMBER PER ANIMAL						
		Colony count per mm³ of specimen				Molars affected	Lesions	Pulp exposures
		Total	Lactobacilli	Streptococci	Staphylococci			
Over 30*	2	4,404	2,029	2,380	0	12.0	12.0	11.0
20–29.9	11	1,694	1,039	656	1	11.1	16.9	5.6
10–19.9	12	1,107	949	122	32	10.2	13.9	3.3
5– 9.9	26	785	590	123	43	8.6	11.4	1.9
1– 4.9	73	520	339	90	63	4.0	5.0	0.3
0.1– 0.9	36	653	424	139	78	1.7	1.8	0
0	19	612	507	81	10	0	0	0

* Caries indices of these two animals: 40.8 and 45.4.

0.5 p.p.m. had moderate numbers of aciduric bacteria with very few molar lesions.

In Table III the oral aciduric bacterial counts are compared to the over-all

caries indices, regardless of the diets ingested by the 179 animals. From caries free hamsters a moderate number of lactobacilli, fewer aciduric streptococci, and very few aciduric staphylococci could be cultured. It seems significant that as the caries indices increased above the 1–4.9 index level, the lactobacillus count also increased by regular increments; the aciduric streptococci also increased but in a less consistent manner, while the aciduric staphylococci decreased in number as the caries indices increased. The high streptococcus count in the 2 animals with caries indices of 40.8 and 45.4 may be related to the modified environment of the large all-inclusive molar lesions which exposed almost every pulp to the oral cavity in these cases.

DISCUSSION

Much evidence has been presented in recent years to show that the microbic flora of the mouth and in particular that of the tooth surfaces is associated in some way with caries activity in man. In the case of induced dental caries in animals, the oral bacterial flora likewise becomes an important environmental factor to be thoroughly studied.

In a systematic investigation of the oral flora in rats with dental lesions induced by dietary means, Harrison (12) demonstrated that the primary aerobic flora of the normal rat tooth includes streptococci (55–60%), staphylococci (14–16%), micrococci, gram positive bacilli (mainly lactobacilli, 15–20% and diphtheroids), gram negative bacilli of the intestinal group, gram-negative cocci, monilia and filamentous forms. This bacterial flora is not unlike that in man. In another report, Harrison (13) pointed out that a progressive increase in the proportion of lactobacilli as well as alpha streptococci occurred in rats developing caries on a coarse particle diet. In animals on a coarse particle diet, into which fluorides and iodoacetate were incorporated with the result that fewer carious lesions were found, there was a definite decrease in numbers of lactobacilli, whereas the total streptococci were increased proportionately. Animals on soft non-caries producing diets showed a moderate percentage of lactobacilli and a higher percentage of acidogenic streptococci, whereas in those on this diet treated with fluorides and iodoacetate there was a marked decrease in lactobacilli and proportionate increase in acidogenic streptococci. In a third report the same investigator (14) indicated that the aerobic flora of large dentinal lesions consists mainly of streptococci. The results of these observations suggested a relationship of lactobacilli with initially developing carious lesions and of streptococci with advanced dentinal lesions.

The hamster has been found to be more suited to studies of dental caries than the rat or other previously used experimental animals because of 3 characteristics: (A) Its molar teeth are susceptible to dental lesions under certain conditions approximating those found in the disease in man. (B) The environmental factors of diet and microbic flora which have been demonstrated to be associated with the development of dental caries in man are similar in the hamster's oral cavity. (C) The lesions produced experimentally in the hamster resemble the lesions of the naturally occurring disease in man.

As has been pointed out previously, in man those portions of the tooth surface on which initial lesions develop are covered by bacterial plaques consisting of filamentous bacteria enmeshing a variety of bacterial forms, predominantly lactobacilli according to Blayney, Kesel, Wach, Bradel, Hemmens and Harrison (6, 15, 16). Stephan (5) has made direct measurements of pH of these plaques in situ and has found pH levels as low as 4.3 in extreme caries-active cases after carbohydrate ingestion. The lactobacilli and other enmeshed microorganisms of the plaque are apparently able to survive and multiply in the low pH which occurs when carbohydrate is available.

The microorganisms collected from the perimolar region of the hamsters were grown on plates of aciduric agar which would permit the growth of only acid tolerating microorganisms. The use of the diminutive aspirator made it possible to collect the microorganisms closely adherent to the molar surfaces. Although it was not found to be feasible to obtain specimens which might be handled as are the bacterial plaques in cultures made from human teeth, it is believed that the method used produced results which are at least roughly comparable to those from plaque culture methods.

It is not unlikely that among the microorganisms cultivated some may have been transients. Possibly the resident flora was contaminated by microbes from the air, food, feces, other hamsters, or the cage. No specific attempt was made to differentiate between the resident and possible transient flora, although the usual bacteriological precautions were taken to prevent extraneous contamination as far as possible in the taking and handling of cultures.

Although there is no evidence bearing on the point, it may be suggested that the surfaces of teeth which are undergoing carious decalcification as the result of acid production by adjacent bacteria would possess a "resident flora" consisting only of acid tolerating microorganisms. In this case the non-aciduric flora, of which large numbers of several species were obtained in the few cultures that were made of that flora, could be considered as transient on the tooth surfaces. This is not advanced as a hypothesis, however, since it is not known whether the surfaces are intermittently or continuously acid in reaction, nor is there evidence that those organisms which do not grow in laboratory media at pH 5.0 cannot subsist in bacterial aggregates on the tooth surface and in food deposits at similar or even lower pH levels.

As the observations on the eruption of the molar teeth indicated, all twelve molars had been subjected to the oral dietary and bacterial environment for the full period of study. During this time dental lesions developed to a much greater degree in animals on certain diet regimens than they did in animals on other diets. Since the hamsters on the deficient diets, i.e., diets that lacked certain portions of the vitamin B components employed in the study, were given the missing factor intraperitoneally, these animals were not deficient from a systemic standpoint in any of the essential dietary factors studied, provided they could assimilate the vitamin component given parenterally, and furthermore there is evidence that animals can do so. Thus the differences in the nutritional factors involved were local differences only. The effects of caries productive

diets differed from the effects of diets producing less caries because of differences in their direct local action in the mouth. Inhibitors in the form of fluorine or possibly other, as yet unrecognized substances contained in the diets apparently minimized the extent of caries developing in animals fed on the commercially prepared diets.

It was found that aciduric bacterial samples from the animals on the different diets varied from one group to another, not in kind, but in quantity of colonies grown. This could be interpreted to mean that all the animals have perimolar flora consisting of essentially the same aciduric, facultatively anaerobic microorganisms. However, under certain favorable oral dietary environments involving the presence or absence of certain vitamin B components, or for some other less evident reason, some bacteria increased to enormous numbers, while under unfavorable conditions such as provided by a high fluorine content in the diet there was no such bacterial increase. In this connection it should be pointed out that although the specific growth requirements have not yet been determined, it is known that oral lactobacilli, in vitro, require carbohydrate for growth; that their optimal pH range is on the acid side of neutrality; and that their growth is much improved when the media are supplemented with liver extract, yeast extract, or tomato—all rich in vitamin B components.

Streptococci, appearing as pin-point colonies on the aciduric plates, could seldom be subcultured and may be less aciduric than the lactobacilli. These streptococci, nevertheless, were found in great numbers in animals with very high caries indices with many pulp exposures, although they were isolated less consistently from animals with less advanced lesions than were the lactobacilli. It is therefore conceivable that these streptococci might have had their source in a somewhat less aciduric flora of the deeper dentinal cavities opening into the pulp chambers.

Although only the aciduric, facultatively anaerobic portion of the perimolar flora was studied at all quantitatively, it seems evident from the data presented that the lactobacilli, considered as a group because of their pleomorphism and mixed fermentation reactions, were the most consistent and numerous organisms found in association with caries in the hamsters studied.

SUMMARY

The oral bacterial flora of the Syrian hamster was investigated. Specimens were obtained from the molar tooth surfaces by means of a small aspirating tube. The specimens were grown on a differential medium having pH of 5.0.

Colonies on a total of 227 acid agar plates, inoculated with the perimolar specimens from 179 hamsters which had been maintained on various diets, were observed and studied. Eight kinds of aciduric microorganisms were isolated: staphylococci, streptococci, and unidentified pleomorphic organisms and 5 varieties of lactobacilli.

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Unsuccessful results have been reported by Arnold, Singleton, and Dean (4) who concluded that single topical applications of 5000 ppm fluoride did not reduce the caries incidence of young adults. East, Ziskin, Stowe, Karshan and Richardson (5) claim no beneficial action on the caries incidence in newly erupted teeth of children subjected to repeated topical applications of potassium fluoride. Bibby (6) reports that no evidence of a reduction in caries activity was found in a 2 year study on the effect of fluoride-containing dentifrices in groups of dental students and orphanage children and refers to data in press in which fluoride mouth washes were unsuccessful.

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diets differed from the effects of diets producing less caries because of differences in their direct local action in the mouth. Inhibitors in the form of fluorine or possibly other, as yet unrecognized substances contained in the diets apparently minimized the extent of caries developing in animals fed on the commercially prepared diets.

It was found that aciduric bacterial samples from the animals on the different diets varied from one group to another, not in kind, but in quantity of colonies grown. This could be interpreted to mean that all the animals have perimolar flora consisting of essentially the same aciduric, facultatively anaerobic microorganisms. However, under certain favorable oral dietary environments involving the presence or absence of certain vitamin B components, or for some other less evident reason, some bacteria increased to enormous numbers, while under unfavorable conditions such as provided by a high fluorine content in the diet there was no such bacterial increase. In this connection it should be pointed out that although the specific growth requirements have not yet been determined, it is known that oral lactobacilli, in vitro, require carbohydrate for growth; that their optimal pH range is on the acid side of neutrality; and that their growth is much improved when the media are supplemented with liver extract, yeast extract, or tomato—all rich in vitamin B components.

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of topical applications of "Septochem" on caries activity in hamsters has been tested (10).

At the time this experiment was undertaken, clinical reports on the effectiveness of urea in reducing the activity of human dental caries had not appeared (11), but the reported effectiveness of urea in counteracting the influence of carbohydrates on the pH of dental plaques (12) suggested that topical urea applications might be effective in reducing caries activity in hamsters.

EXPERIMENTAL

All hamsters were obtained from an inbred colony maintained on a stock diet of Purina Rabbit Checkers. From 20 litters between 4 and 5 weeks old, 100 animals were distributed at random into 5 groups of 19 to 21 animals each.

Group I animals were maintained on a diet of whole dry yellow corn to study the effect of this ration on caries activity. The results of this experiment are reported elsewhere.

Group II animals (12 females and 8 males) received the standard Hoppert-Webber-Canniff diet (13): corn meal 60%; powdered milk 30%; linseed meal 6%; alfalfa meal 3%; and NaCl 1%. The corn meal was of a semi-fine grind and all of it passed a 20-mesh screen. The fluoride content of the ration analysed by the method of Willard and Winter (14) was 0.87 ppm fluorine.

Group III animals (12 females and 8 male) received a diet containing 2 parts of the H-W-C mixture and 1 part of confectioners sugar. The fluoride content of this mixture was 0.92 ppm fluorine.

Group IV animals (13 female and 8 male) received the same ration as Group II. The molar teeth of hamsters in this group were treated for 2 minutes once a week with topical applications of a NaF solution containing 1000 ppm fluorine.

Group V animals (12 female and 8 male) received the same H.W.C.-sugar ration as Group III. The molar teeth of these animals were treated for 2 minutes each week with a 50% urea solution.

The method of treatment in Groups IV and V was as follows: Each animal was lightly anesthetized in a desiccating jar containing a small amount of ether in the bottom. The anesthetized animal was placed supine on a flat board having a spring prop to hold open the mouth. From a 22 gauge needle in a 10 cc Leur Lock syringe the solutions were dropped continuously over the upper teeth for 1 minute. The excess fluid was aspirated from the mouth by means of a 14 gauge needle attached by rubber tubing to a water vacuum pump. The lower teeth were treated by removing the animal from the board, propping open the mouth with a small pair of forceps placed in the diastema between incisors and molars, and by dropping the solution over the teeth and aspirating excess fluid. This was done several times during the 1 minute period. The total treatment time was 2 minutes for each animal. At the end of the treatment all excess fluid was carefully aspirated, but the mouth was not rinsed. At no time during the treatment were the needles brought into contact with the tooth surfaces.

Animals were kept in metal cages with wood shavings for bedding. Diets and distilled water were available ad libitum. Supplements of fresh alfalfa

were supplied to each group once a week. The animals were maintained on this regime for 110 days, after which time they were sacrificed. From heads fixed in 10% formalin, the jaws were removed, stripped of soft tissue, and dried. The teeth were examined under a low-power binocular microscope (2.0 × 10), and all gross lesions were charted and scored by a method previously described (15).

TABLE I

Caries Incidence as Found in Individual Molars

MOLARS	GROUP II H.W.C. DIET		GROUP IV H.W.C. DIET, F TREATED		GROUP III H.W.C. SUGAR		GROUP V H.W.C. SUGAR UREA TREATED	
	% Molars Affected	Ave. % of Tooth Decayed	% Molars Affected	Ave. % of Tooth Decayed	% Molars Affected	Ave. % of Tooth Decayed	% Molars Affected	Ave. % of Tooth Decayed
Female								
Maxillary								
I	0	0	0	0	62.5	6.1	100	19.9
II	4.3	0.09	0	0	75.0	34.8	100	59.7
III	0	0	0	0	83.5	45.4	100	69.0
Mandibular								
I	0	0	0	0	62.5	6.4	96	17.5
II	33.3	0.4	30.8	12.2	79.1	17.2	100	48.3
III	0	0	3.8	0.04	54.1	7.7	100	33.0
Male								
Maxillary								
I	0	0	0	0	56.2	5.2	100	29.6
II	21.4	2.8	0	0	62.5	31.1	100	81.8
III	71.5	10.0	6.3	2.1	87.5	59.5	100	99.0
Mandibular								
I	7.2	3.6	0	0	62.5	20.1	89	47.8
II	93.0	25.1	50.0	5.1	68.8	10.7	100	60.4
III	35.7	1.2	0	0	68.8	21.7	94	62.5

RESULTS

The animals showed no unfavorable effects or retardation in growth due to the treatment[2]. They remained in good health except for a few days when an epidemic of "sniffles" passed through the colony.

The caries experience of the treated and non-treated groups is indicated in Table I. From these data the following interpretation is made: 1. Fluoride treatment seemed to offer some protection for male animals but not for female. 2. The urea treated animals had a much higher caries experience than animals which received no treatment at all. 3. The addition of sugar to the H.W.C.

[2] One female in Group V died from accidental over anesthetization. The caries experience of this animal is not included in the final results.

ration increased the caries producing properties of the diet to a considerable degree. 4. In groups II and IV male animals experienced greater caries activity than female.

DISCUSSION

In most of the previous experiments on the effectiveness of topical fluoride applications in reducing caries activity, treated teeth in one quadrant have been compared to untreated teeth in an adjacent quadrant of the same mouth (1, 2, 5, 6). One recognizes that better comparisons and more uniform control can be obtained by this method; however, due to the extremely small size of hamsters and the technical difficulties involved in such procedures, repeated treatment of isolated quadrants was not considered practical. Comparison of individual animals is believed to be satisfactory if experimental procedures are carefully controlled and the groups are sufficiently large. The suggestion is not made that either was adequate in this experiment.

Female animals on the H.W.C. diet (Groups II and IV) were less susceptible to dental caries than males on the same ration, with the result that the difference between fluoride-treated and non-treated females was too small for satisfactory comparisons. For males, however, the fluoride applications seemed to offer slight protection as evidenced by a reduction in the number of teeth affected and the extent of cavitation.

The mechanism whereby urea reduces caries activity is attributed mainly to the liberation of ammonium carbonate which neutralizes the acids produced in dental plaques (16). By direct measurement of pH values of dental plaques in situ, Stephan (12) reported that 40 to 50% urea applied for 1 to 5 minutes exerts a marked inhibitory effect on the pH drop produced by glucose for as long as 24 hours after the urea applications. Since the caries inhibitory effect of urea solutions lasts only as long as sufficient ammonium carbonate is present in the plaque to neutralize the acid effect of carbohydrates, the beneficial effect of weekly 2-minute treatments was questionable. However, it was thought that some evidence of caries inhibition might be detected.

The great increase in caries activity in animals that received urea treatment was unexpected and remains unexplained. Since control groups were not treated with distilled water, it is impossible to conclude, at this time, that the urea was a factor in the process. The possibility exists that the treatment procedure itself or other unrecognized factors were conducive to an increase in caries activity.

If the reaction of the animals to the treatments favored an increase in caries activity, perhaps there was a greater degree of protection than is apparent from these data. Both the fluoride and the urea may have exerted more caries inhibitory action than can be recognized. Unfortunately neither additional animals nor facilities were available for running more control groups.

SUMMARY AND CONCLUSIONS

In a group of hamsters on the Hoppert-Webber-Canniff diet, weekly 2-minute treatments with topical applications of NaF solution containing 1000 ppm

fluoride apparently afforded slight protection for males. The difference between treated and untreated females was too small to permit satisfactory comparisons.

Another group of hamsters receiving a H.W.C.-sugar ration was not benefited by weekly 2-minute treatment with a 50% urea solution, when compared to untreated animals on a similar diet.

Caries activity was increased by the addition of sugar to the H.W.C. formula, and a sex difference was noted in some groups.

The Syrian hamster appears to be suited for experimental studies on the caries inhibiting action of topically applied drugs. However, technical difficulties necessitate very careful control of all experimental conditions. The results reported suggest interesting possibilities and indicate that additional work needs to be done to determine the mechanisms associated with caries activity in this species.

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VARIATIONS IN COUNTS OF LACTOBACILLI MADE FROM SINGLE SPECIMENS OF SALIVA[1]

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Various sources of error are responsible for inaccuracies in counts of salivary lactobacilli. The first error is introduced when paraffin is chewed and the stimulated saliva deposited in a bottle. Hadley (6) found it impossible to obtain uniform counts from an individual, even on successive days, due to the fact that "paraffin chewing cannot regularly dislodge the same number of organisms from the teeth." A second source of error may be variation in the lapse of time between awakening of the individual and collection of the specimen. If saliva is collected immediately upon awakening, there may be conceivably less salivary flow, and a greater concentration of bacterial content, than if the salivary glands have been reflexively stimulated by the odor of food.

Several sources of error in making agar plate counts from samples of fluids have been listed (1, 2): 1, failure of some bacteria to grow because of incubation temperature, medium, etc.; 2, inaccuracies in measurement of material; 3, mistakes in counting, computation and recording; 4, contamination; and 5, the uneven distribution of the bacterial population in the original substance. "Bacteria in milk and cream usually occur in groups of from two to several hundred individuals. The customary shaking of samples and dilutions is an inadequate method of breaking apart the clumps, so that, under optimum conditions, the count of colonies on the plate represents the number of isolated individuals and groups of two or more bacteria . . . .This error would not be troublesome if the groups were of constant average size; but observations indicate . . . they contain an average of 2 to 6 cells, while unusual samples . . . may have groups of 25 or more . . . . " (1). The first 4 of these sources of error in making agar plate counts were carefully eliminated in collecting the data here presented. We attempted, by various means, to overcome the clumping of the bacteria.

Other workers have made efforts to eliminate clumping. Crowley and Rickert (4) attempted to break up bacterial clumps by spraying the dilution through an atomizer for 30 minutes. They found that such a method gave consistently higher counts but bacterial clumps still were present. A dilute solution of NaOH used as a diluent did not eliminate clumps. They reported that counts taken on the same individual at different times of day varied greatly.

Bibby and Maurer (3) found a difference in the counts on plates made from different dilutions of saliva, and believed that agglutination of organisms might

[1] Presented at the 24th General Meeting of the International Association for Dental Research, Kansas City, Missouri, March 16 and 17, 1946 (*J. D. Res.*, **25**: 167, 1946). Received for publication June 12, 1946.

be influencing the results. They reported that of the various methods used to reduce the spontaneous agglutination of salivary organisms, the addition of 1% horse serum to standing saliva or saliva dilution tubes gave satisfactory results. No specific data on counts were given.

Various workers (7, 10, 11, 15, 16) have recognized the fallacy of basing a diagnosis of caries activity on a single plate count of lactobacilli. Some workers (5, 10, 13) have attributed the variations obtained in counts made on successive days to actual variations in the population of oral lactobacilli. Ferier and Leonard (5) described what they called the diurnal tide of bacteria in the mouth. Smolelis (14) reported the effects of time and temperature on the lactobacillus counts.

Snyder (16) stated that errors occur in plate counts, and indicated the necessity of successive studies of individuals rather than a single specimen of saliva. In correlating his colorimetric method for the estimation of relative numbers of lactobacilli in saliva with plate counts on the same sample, Snyder found it possible to select by negative color change with a 4% error, those specimens having less than 1000 lactobacilli per cc. It became increasingly difficult to correlate color changes with higher lactobacillus counts.

PROCEDURE

With the initiation of a Caries Control Laboratory based on the system set up by Jay (8), tests were made of our ability to obtain consistent results. A number of plates were spread from a single specimen of saliva. The wide variation in the counts obtained when 8 or 10 plates were made from the same saliva sample suggested serious error in the manipulation of the materials. After carefully checking for all such possible sources of error, similar discrepancies were found in counts from plates made not only from the same dilution of a sample, but from the same 1 cc. pipetful of this dilution when 0.1 cc. was placed on each of a series of plates.

The agar used was the Hadley modification of Kulp's tomato agar (6). The diluent was the beef infusion broth, pH 5, used by Jay (8). The original saliva specimen was thoroughly shaken; a sterile 1 cc. pipet was used to place 1 cc. of the specimen into a known amount of beef broth. The saliva and diluent were mixed by drawing in and out of the pipet at least 10 times; 0.1 cc. of the mixture was placed on a tomato agar plate; this was distributed evenly with a sterile L-shaped glass rod spreader, of uniform size in each instance. The plates were incubated for 96 hours. The lactobacillus colonies were counted and computations made to estimate the number of organisms per cc. of saliva. Most samples were allowed to stand 24 hours before plating. None were plated in less than 6 hours after collection of the saliva.

Five steps were taken in the attempt to reduce the error in the plate counts:

1. Before starting to collect these data, the procedure was checked carefully for possible errors in measurement. The considerable amount of foam which adhered to the outside of the pipet when saliva was taken from the original sample was routinely removed with sterile gauze. 2. Several methods of mixing the original specimen were employed in efforts to attain an equal distribution of bac-

teria. These methods were: (a) shaking the specimen vigorously by hand for 3 minutes; (b) shaking for 1 minute on an amalgam mixer (Wig-L-Bug) which had been modified to carry the specimen bottle in a cylinder; (c) beating specimen on a high speed stirrer, using 3 beaters each having a different arrangement of blades; (d) shaking on a Kahn shaker for 10 minutes. 3. Thorough mixing of saliva and diluent were sought by: (a) drawing the mixture in and out of a pipet at least 10 times; or (b) placing a sterile rubber stopper in the test tube and shaking the tube for 3 minutes before plating. 4. In some instances, a large portion of the shaken original sample was placed in a diluent, to overcome the variation in counts caused by removing several small portions, each containing unknown numbers of clumps of bacteria. This large portion of saliva and the diluent were beaten with the high speed stirrer to mix thoroughly, and, if possible, to break up clumps of organisms. From this mixture, several 1 cc. portions were removed and placed in a second diluent for plating. 5. Different diluents were used to determine whether or not a change of diluent would permit a better distribution of organisms. The beef broth was replaced in some instances by distilled water, N saline solution, or fluid agar (1 gram to 400 cc. of distilled water).

DATA

The data presented show the variations obtained in series of plate counts made from single saliva specimens when, 1, various methods of mixing were employed, and, 2, when different diluents were used. The data also demonstrate trends in the counts of the individuals tested.

In tables I and II the methods used for mixing the saliva, and the diluent used, are indicated in the first column. Column 2 shows the number of plates made from each specimen. A separate dilution was used for each plate, and the original specimen was shaken in the designated manner before each dilution was made. The mean was computed for each series of plates. In order to obtain a figure for each specimen that could be compared with a similar figure for all other specimens, the range $\pm\frac{1}{2}$ the mean was arbitrarily chosen. In column 3 is recorded the percentage of plates in each series whose counts were within the range $\pm\frac{1}{2}$ the mean for that series. The highest and lowest counts obtained in each series are recorded in column 4.

Jay's system of diagnosis of caries activity is based on grouping the counts into 5 classes.[2] The percentage of plates of each series which fall into each of these classes is given in columns 5, 6, 7, 8, 9.

The method of mixing used for data shown in Table I varies. One cc. of saliva was placed in a known amount of beef broth. One-tenth cc. of this mixture was plated. Group A (column 1) is made up of those specimens treated in the manner originally intended for use in our Caries Control Laboratory. The specimens were shaken by hand for 3 minutes. Dilution was made, and saliva and diluent mixed by drawing in and out of the pipet at least 10 times. Group B specimens were shaken on an amalgam mixer (Wig-L-Bug) for 1 minute. Broth and saliva

[2] Jay's classes are as follows: ± = 0-1000; + = 1000-5000; ++ = 5000-10,000; +++ = 10,000-50,000; ++++ = over 50,000.

TABLE I

Effects of different methods of mixing on reproducibility of counts

METHOD	NO. PLATES	% OF PLATES WITHIN ±½ MEAN	RANGE	% WITHIN ± CLASS	% WITHIN + CLASS	% WITHIN ++ CLASS	% WITHIN +++ CLASS	% WITHIN ++++ CLASS
A	10	100	12,700– 27,100				100	
	19	75	5,100– 31,400			30	70	
	9	100	1,300– 2,700		100			
	26	81	750– 6,700	8	88	4		
	27	41	6,000–249,000			11	55	34
	13	92	15,650– 51,700				92	8
B	10	90	3,990– 13,680		30	60	10	
	10	90	6,460– 19,570			70	30	
	5	100	10,450– 16,680				100	
	6	83	7,220– 19,950			50	50	
	8	13	350– 7,800	87		13		
	9	33	1,200– 20,600		78		22	
	8	88	4,100– 12,600		13	62	25	
C	10	50	950– 5,320	10	80	10		
	9	77	5,700– 18,620			45	55	
	6	100	14,630– 21,390				100	
	6	100	8,930– 16,340			17	83	
	10	30	0– 550	100				
	10	100	6,200– 15,100			30	70	
	9	78	4,000– 17,300		45	33	22	
D	8	62	190– 1,900	62	38			
	8	100	3,300– 6,300		25	75		
	10	100	45,600– 74,860				30	70
F	10	80	2,280– 9,690		50	50		
	9	66	800– 8,300	11	55	34		
	10	100	17,200– 37,000				100	
G	10	70	15,200–++++*				70	30
	8	12	1,100– 23,100		63	12	25	
	10	50	40,300–181,700				10	90
H	8	50	200– 8,700	12	76	12		
	8	100	78,000– 96,000					100
	9	44	2,850– 64,690		33	22	33	12
I	8	75	380– 2,470	62	38			
	9	22	1,330– 45,810		55	22	23	
J	8	75	380– 2,850	38	62			
	9	77	1,710– 8,360		77	23		

* Impossible to count accurately.

were mixed by drawing in and out of the pipet. Group C specimens were beaten on the high speed stirrer with beater # 1 for 2 minutes. Saliva and diluent were

mixed by drawing in and out of the pipet. Group D specimens were beaten with beater #1, and the saliva and diluent were mixed by placing a sterile rubber stopper in the test tube and shaking vigorously by hand for 3 minutes. Group E specimens were beaten with beater #2 for 2 minutes, and saliva and diluent mixed with pipet. Group F specimens were beaten with beater #2, and saliva and diluent mixed by shaking. Group G specimens were beaten with beater

TABLE II

Effects of different diluents on reproducibility of counts

METHOD	NO. PLATES	% OF PLATES WITHIN ±½ MEAN	RANGE	% WITHIN ± CLASS	% WITHIN + CLASS	% WITHIN ++ CLASS	% WITHIN +++ CLASS	% WITHIN ++++ CLASS
K	8	75	500– 1,800	25	75			
	8	37	500–++*	25	37	38		
	8	100	96,000–120,000					100
	8	100	96,000–120,000					100
	8	100	1,400– 2,500		100			
	8	100	2,100– 3,700		100			
L	8	75	72,000–276,000					100
	7	100	96,000–144,000					100
	8	87	15,600–++++*				87	13
M	8	100	336,000–588,000					100
	7	100	336,000–444,000					100
	8	87	8,000– 15,000				100	
	6	100	6,000– 12,400			67	33	
	8	100	10,900– 20,400				100	
	8	100	7,000– 15,500			37	63	
	7	100	700– 1,700	43	57			
N	8	100	264,000–408,000					100
	8	100	276,000–360,000					100
	7	57	1,000– 4,600		100			
	8	50	800– 6,700	12	63	25		
	8	100	46,800– 58,000				50	50
O	8	87	27,400– 90,400				63	37
	8	75	26,400–++++*				63	37
	8	50	600– 4,700	25	75			
	6	83	1,100– 2,500		100			

* Impossible to count accurately.

#3, and saliva and diluent mixed with a pipet. Group H specimens were beaten with beater #3, and saliva and diluent mixed by shaking. Group I specimens were shaken on a Kahn tube shaker for 10 minutes, and saliva and diluent mixed with a pipet. Group J specimens were shaken on a Kahn tube shaker for 10 minutes, and saliva and diluent mixed by shaking.

It is shown from the data in table I that none of these methods of mixing the saliva specimens produced satisfactory reduction of range in all series. It is also apparent that results obtained when the saliva and diluent were mixed by shaking

by hand (D, F, H, J) were as variable as when the mixing was done by drawing the fluid in and out of a pipet (A, B, C, E, G, I).

Of the 39 series of plates made, using various methods of mixing the original sample and using beef broth as a diluent, 26% (10 series) had 100% of the plates within the range of $\pm\frac{1}{2}$ the mean. Twenty-eight percent (11 series) had 50% or less of the plates within $\pm\frac{1}{2}$ the mean.

Table II shows the results of removing a larger portion of saliva from the original sample, and making 2 dilutions of the original specimen, and of varying the diluent. Each specimen was first shaken for 5 minutes on a Kahn tube shaker and then 5 cc. of it were removed to 5 cc. of the first diluent. This was done because removing 5 cc. from the original specimen should give a more accurate sample than removing the 1 cc. previously used, assuming clumping of the bacteria in the saliva. This 10 cc. mixture was then beaten for 2 minutes on the high speed stirrer, with the purpose of breaking up clumps of organisms if possible. One cc. of the mixture was then placed in a known amount of the second diluent, a sterile stopper placed in the test tube, and the tube shaken vigorously by hand for 3 minutes. One-tenth cc. was then plated. Fluid agar was used as the first diluent in some series because of its viscosity. In other series, normal saline or distilled water was used. The second diluents were beef broth, normal saline, fluid agar or distilled water. A different second dilution from the single first dilution was made for each plate. The first dilution was beaten with the high speed stirrer before removing 1 cc. for each second dilution. Group K specimens were diluted first with fluid agar, and second with beef broth. Group L specimens were diluted first with fluid agar, and second with normal saline. Group M specimens were diluted first with normal saline, and second with normal saline. Group O specimens were diluted first with distilled water, and second with distilled water.

Here are seen the results of removing a larger portion of the original sample to a diluent, and then making a second dilution from this. Of the 25 series made, 56% (14 series) had 100% of the plates within the range $\pm\frac{1}{2}$ the mean. Twelve percent of the series (3 series) had 50% or less of the plates within the range $\pm\frac{1}{2}$ the mean.

If the 16 series of plates in which fluid agar was used as a first diluent, (K, L, M) are considered as a group, 69% (11 of 16) had 100% of the plates within the range of $\pm\frac{1}{2}$ the mean. Only 6% (1 of 16) had 50% or less of the plates within this range.

DISCUSSION

The wide variations in counts obtained when a series of plates is made from a single saliva specimen, added to the admitted lack of uniformity in collecting the specimens, may raise doubt as to the value of counts of lactobacilli in saliva as a test for caries activity.

To obtain a picture of the lactobacillus counts of an individual over a period of time, graphs were made to show the various series grouped according to the individuals from whom they were taken. Along the lower border of *figs. 1–4*, the

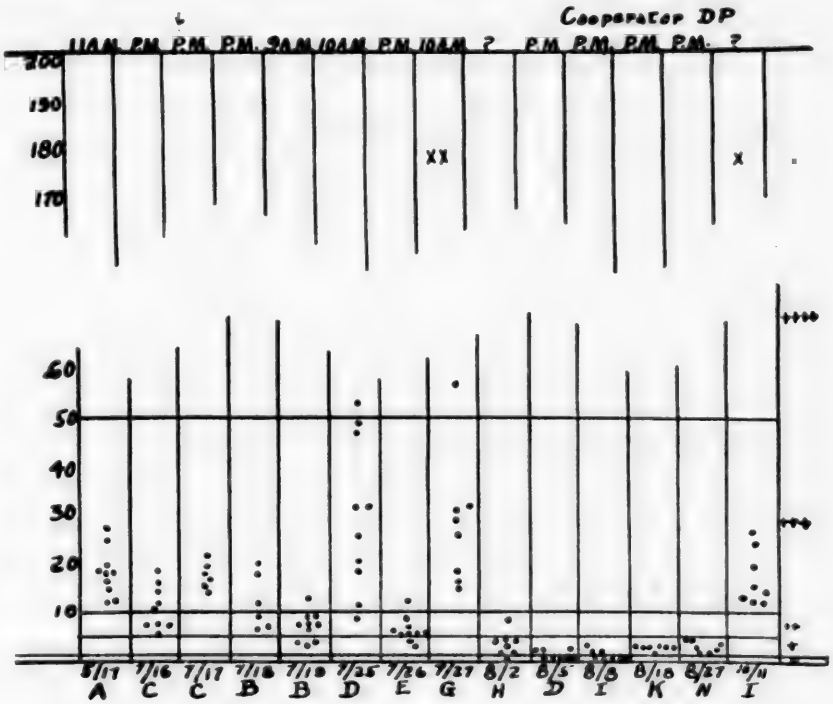

Fig. 1

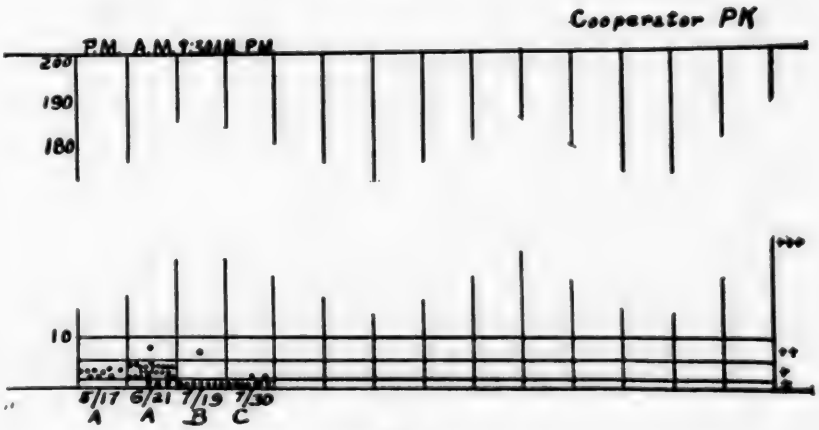

Fig. 2

letters indicate the manner of treating each sample, and the figures indicate the date the specimen was collected. The numerals along the ordinate represent the estimated number, in thousands, of lactobacilli per cc. of saliva as shown by the

count of colonies on each plate. Jay's grouping into classes is indicated along the ordinate on the right side of the figure. The samples were not collected at the same time each day. Some of the differences in counts from different specimens

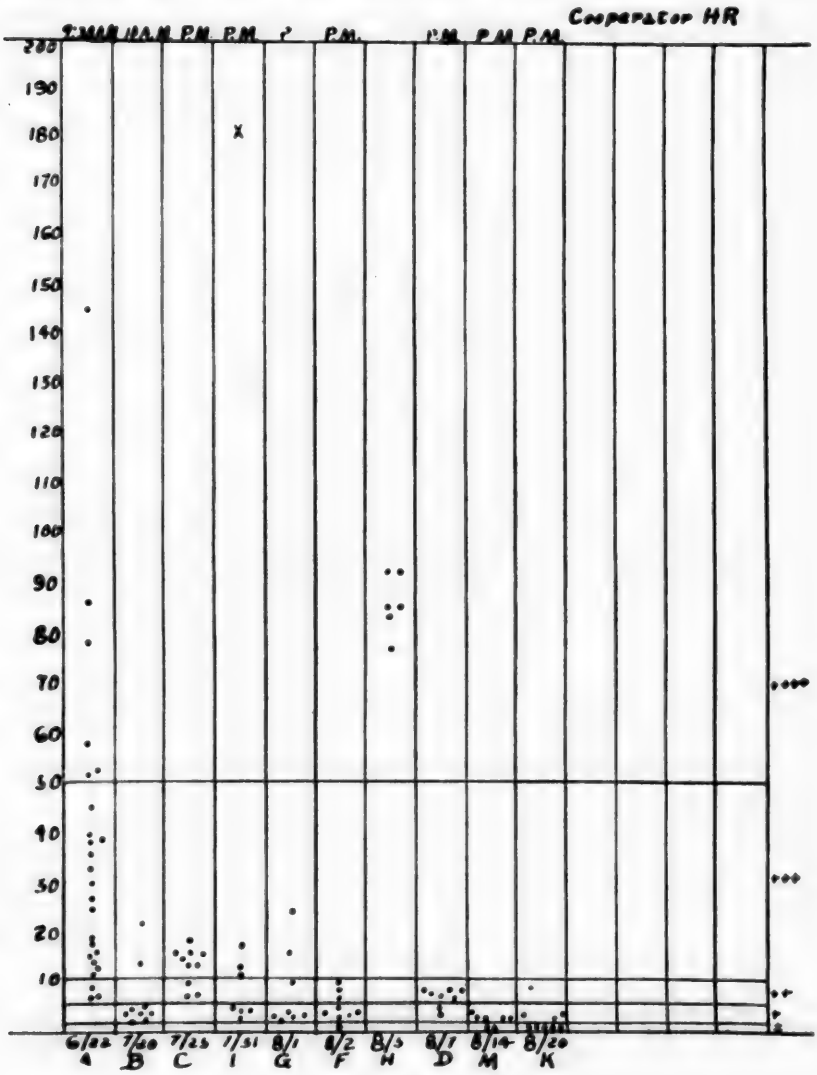

Fig. 3

may be due to this fact. The time of collection is indicated along the top of the graph.

Cooperator DP (*fig. 1*) had counts fairly consistent in ranges ++ and +++ for May and July; fell to ± and + for August, and rose, according to a single

sample, to +++ in October. Cooperator PK (*fig. 2*) remained ± and +, and eventually dropped to a consistent negative and was discontinued for this investigation. Cooperator HR (*fig. 3*), during June and July, had a number of plates

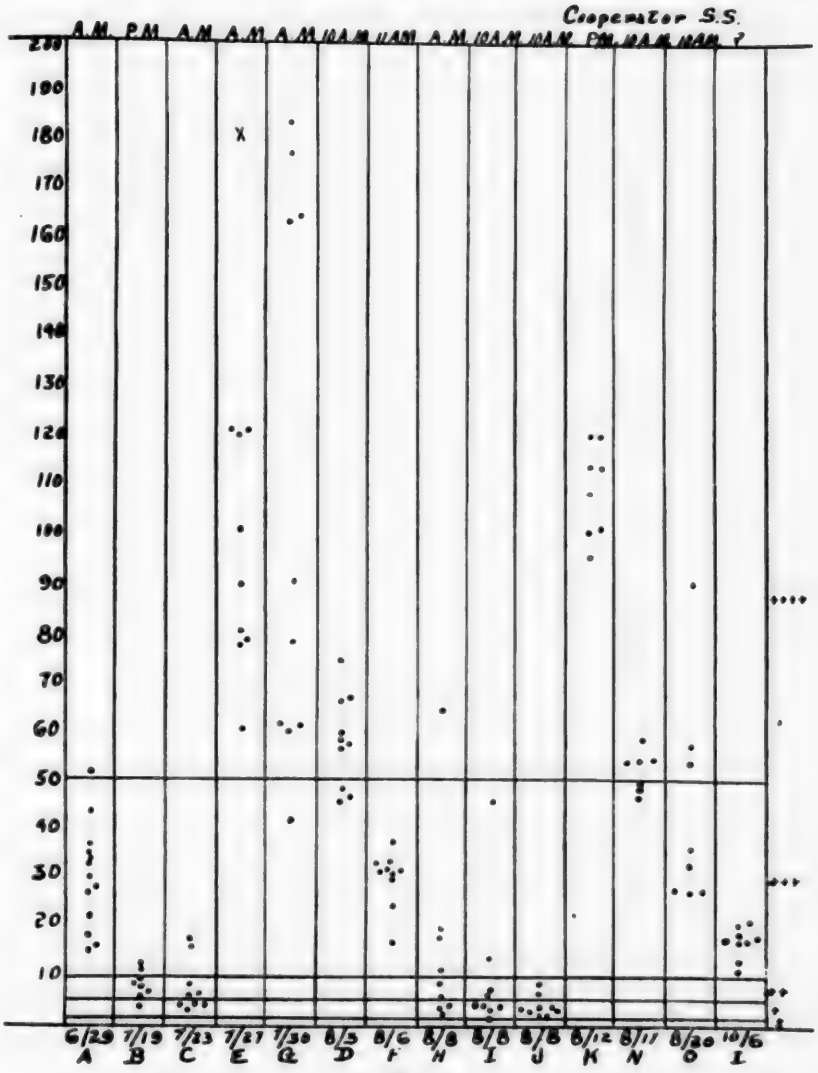

Fig. 4

in the +++ range, and later dropped to ± and + in August, with the exception of a rise in a single specimen in August to ++++. Cooperator SS (*fig. 4*) had plates conspicuously spread over the entire graph, but showed a large number in the ranges +++ and ++++. However, the samples taken on 7/19 and 8/8

considered alone, would indicate much lower counts than the others. Cooperator JM (*fig. 5*) had ++ and +++ counts for 8/21 and 8/22, and then the counts

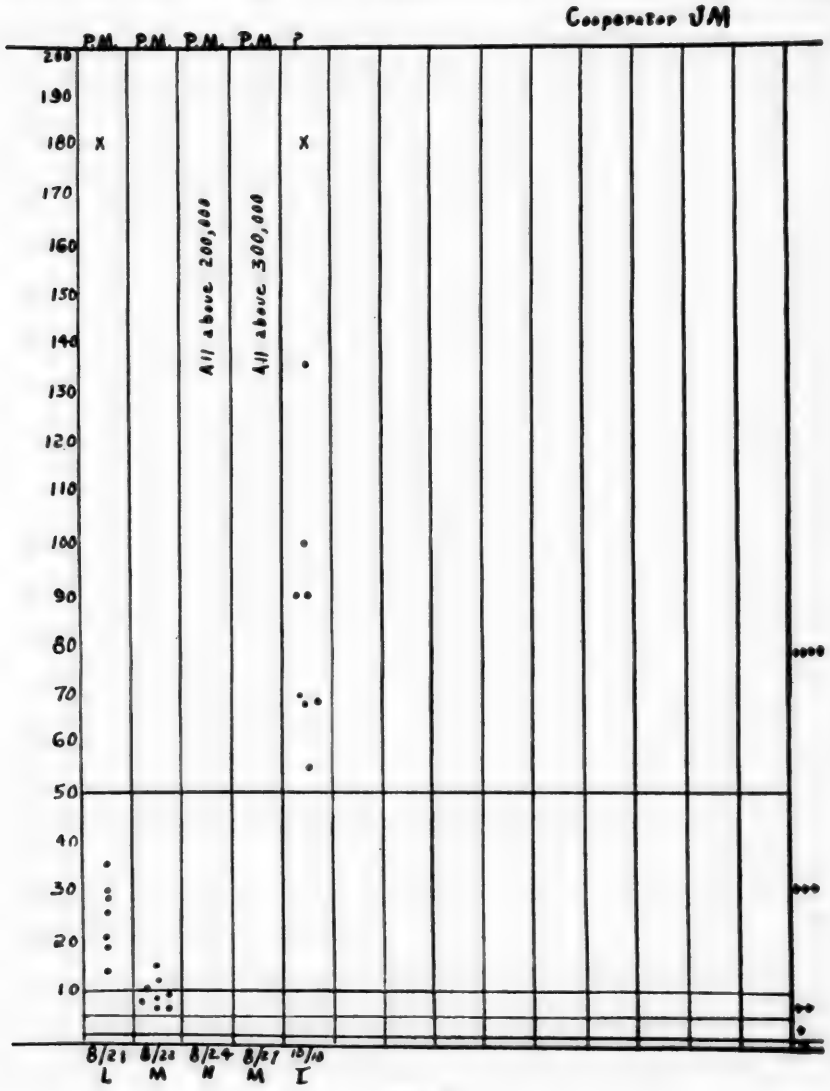

Fig. 5

rose to a point so high that the colonies could not be accurately counted on some plates with the dilution used. An X at the 180,000 mark on any graph indicates a plate bearing colonies too numerous for accurate count. In all cases, this number would be above 200,000.

The range of variations of plate counts on a single specimen is brought out clearly by the graphs, and demonstrates what misconceptions can arise from counting a single plate, or a small number of plates from a single specimen. The apparent lack of correlation between the color method of Snyder, and Jay's colony count (16) may be accounted for on this basis.

CONCLUSIONS

No method of shaking or beating the original saliva specimen before removing 1 cc. to beef broth diluent produced plate counts with a relatively narrow range in all series. When the variation in each series of plates was measured by computing the number of plates of the series which had counts within the range of $\pm\frac{1}{2}$ the mean for that series, it was found that:

26% of the series had 100% of the plates within the range $\pm\frac{1}{2}$ the mean.

28% of the series had 50% or less of the plates within $\pm\frac{1}{2}$ the mean. Shaking the saliva and diluent by hand gave no more consistent results than mixing with a pipet.

Results were more consistent in those series where the original sample was shaken and 5 cc. removed to 5 cc. of fluid agar for the first dilution. This mixture was beaten with the high speed stirrer, and a second dilution made. Varying the second diluent did not improve the results. Computations show that by this method:

69% of the series had 100% of the plates within the range $\pm\frac{1}{2}$ the mean.

6% of the series had 50% or less of the plates within $\pm\frac{1}{2}$ the mean. With any method used, some unsatisfactory results were obtained.

Graphs for each cooperator of series of plates made at intervals over a period of time show that general trends exist, and that an individual can be diagnosed as having low or high caries activity according to the classification of Jay. However, consideration of a small number of single counts, or a small number of series of counts, can lead to erroneous conclusions.

SUMMARY

Series of plates, each made from single specimens of saliva, with extreme care in measurement, gave colony counts which varied over wide ranges. Different methods of mixing the original specimens were employed in an effort to secure better distribution of the organisms. Hand shaking, an amalgam mixer, a Kahn tube shaker, and 3 types of beaters were used. None of these increased the uniformity of counts. More consistent results were obtained when 5 cc. of the original specimen were placed in an equal amount of fluid agar and beaten before a second dilution was made and the specimen plated. All methods used gave some series showing wide variations.

The assemblage of the data from each cooperator emphasized the wide range of counts obtainable from a single specimen. It is obvious that, while trends are apparent in the lactobacillus counts of individuals, conclusions as to hourly or daily fluctuations are unjustified when based on single counts or series of counts such as used in this study.

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SALIVA INCUBATED WITH GLUCOSE AS A REPLACEMENT FOR TRYPTOPHANE IN A SYNTHETIC MEDIUM SUPPORTING THE PRODUCTION OF ACID BY LACTOBACILLUS ACIDOPHILUS[1]

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An oral strain of *Lactobacillus acidophilus* has been grown upon a synthetic medium in which human saliva hydrolyzed by acid or alkali had been substituted for a casein hydrolyzate (1). It did not grow satisfactorily when unhydrolyzed saliva concentrated as much as 7 fold was used as the substitute (2). These observations indicated that whole saliva is a potential source of the major nutrients, presumably the amino acids, present in the casein hydrolyzate. Consequently it became of interest to determine whether or not mechanisms exist in saliva itself whereby amino acids may be set free from proteins and whether these can be made available for supporting bacterial growth. Observations made during the investigation of these problems are reported here.

In the first section of this study whole saliva just as collected was incubated for various periods without further treatment and then substituted for (a) the added tryptophane of (b) the casein hydrolyzate of the basic medium. Observations upon the quantity of acid set free during the growth of the test organism in the media with such substitutions were made. In another series of experiments the saliva was incubated with glucose before being added to the test media. Then portions of saliva so treated and incubated were used as replacements in the synthetic media. The amount of acid produced was significantly larger in the second series. The experimental procedures and data follow.

EXPERIMENTAL

Saliva Treatment. Saliva was collected from 10 normal persons. Paraffin was chewed to stimulate the secretion of the fluid. The specimens whose volumes totaled 225 ml. were pooled without further treatment for the experiments of the first series. Aliquots were withdrawn for a replacement test experiment and for chemical analyses. The remainder was incubated at 37.5°C., for 192 hours. At the intervals shown in the tables additional aliquots were withdrawn for other replacement tests. In the second series a similar quantity of pooled saliva was mixed with an autoclaved solution of glucose sufficient to produce a 1% concentration of the sugar. Aliquots of the saliva containing glucose were taken and treated in a manner similar to that described for the first series.

Methods.—Saliva, whose secretion was stimulated by chewing paraffin wax was collected from 10 individuals. The samples were pooled. A 225 ml. aliquot of the pooled saliva was incubated at 37.5°C. for a total of 192 hours. Samples of

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the saliva were withdrawn for the replacement experiments at the beginning of the incubation and at various intervals thereafter (see tables).

Media.—The media were prepared in accordance with procedures described previously (2). The basic medium contained all substances essential for the growth of the organism. In preparing some lots either (a) tryptophane or (b) casein hydrolyzate or (c) both constituents were omitted in order that substitution of incubated saliva might be made. In arranging these substitutions 5 ml. of a double strength medium having appropriate omission was placed in a tube. Then 3 ml. of the saliva being tested was added, the pH was adjusted to 6.8 and the volume was made up to 10 ml. with distilled water. The tubes were plugged and autoclaved at 10 pounds pressure for 10 minutes.

Tests for Growth. The test micro-organism was *Lactobacillus acidophilus* strain 333. The nutritional requirements of this organism have been described previously (3). Duplicate tubes were inoculated with 0.1 ml. of a suspension of the lactobacillus. The tubes were then incubated for 72 hours at 37.5°C. when the acid formed was titrated with the Coleman electrometer to pH 6.8.

RESULTS

When tryptophane was omitted from the basic medium and replaced with freshly collected saliva and the mixture incubated with *Lactobacillus acidophilus* no significant production of acid occurred. When the replacement was saliva which had been incubated at 37.5°C. for some hours there was some production of acid. This quantity was larger with saliva incubated for 24 hours and declined somewhat with that which was incubated for 192 hours. Similar observations were obtained when saliva was used as a replacement for casein hydrolyzate only or for tryptophane plus casein hydrolyzate. Data for experiments in this series are recorded in Table I.

The data obtained when saliva which had been incubated *with glucose* was substituted for (a) tryptophane, (b) casein hydrolyzate or (c) for both are recorded in Table II. These observations show that saliva incubated with glucose is a very satisfactory replacement for the tryptophane of the synthetic medium. It was much less effective as a substitute for casein hydrolyzate, though considerably more efficient than saliva which had been incubated without sugar present. When both casein hydrolyzate and tryptophane were omitted from the medium and replaced by saliva incubated with glucose the amount of acid produced by the organism was approximately the same as when only the casein hydrolyzate was omitted.

We have shown previously (1) that saliva *hydrolyzed* with alkali can be substituted for tryptophane in a synthetic medium. The data of Table I and II provide convincing evidence that this amino acid is likewise made available *by incubating* saliva with glucose and is not available in saliva incubated alone. In a subsequent communication we will present additional evidence in which a procedure totally different is used to demonstrate that *free* amino acids increase in quantity during incubation with glucose. When glucose is not added the *free* amino acids decrease in concentration.

TABLE I

*Acid produced in basic medium in which incubated saliva (3.0 ml.) was substituted for certain essential growth factors**

MEDIUM	ESSENTIAL SUBSTANCES OMITTED	PERIOD OF INCUBATION OF SALIVA REPLACEMENT	ACID PRODUCED
		hrs.	*ml.*
Basic synthetic medium†.....	None	No saliva replacement	5.90‡
Basic medium with omissions.....	Tryptophane	0	0.00
Basic medium with omissions.....	Tryptophane	24	0.40
Basic medium with omissions.....	Tryptophane	192	0.18
Basic medium with omissions.....	Casein Hydrolyzate	0	0.00
Basic medium with omissions.....	Casein Hydrolyzate	24	0.27
Basic medium with omissions.....	Casein Hydrolyzate	192	0.06
Basic medium with omissions.....	Tryptophane plus Casein Hydrolyzate	0	0.00
Basic medium with omissions.....	Tryptophane plus Casein Hydrolyzate	24	0.17
Basic medium with omissions.....	Tryptophane plus Casein Hydrolyzate	192	0.00
Basic synthetic medium.....	None	192	5.62§

* All media inoculated with 0.1 ml. of suspension of Lactobacillus strain 333.
† See reference (3).
‡ Control for growth of organism.
§ Control for inhibition by incubated saliva.

TABLE II

*Acid produced in basic medium in which incubated saliva plus glucose (3.0 ml.) was substituted for certain essential growth factors**

MEDIUM	ESSENTIAL SUBSTANCES OMITTED	PERIOD OF INCUBATION OF SALIVA REPLACEMENT	ACID PRODUCED
		hrs.	*ml.*
Basic synthetic medium†.....	None	No saliva present	5.90‡
Basic medium with omissions.....	Tryptophane	0	0.00
Basic medium with omissions.....	Tryptophane	24	3.46
Basic medium with omissions.....	Tryptophane	192	5.00
Basic medium with omissions.....	Casein Hydrolyzate	0	0.00
Basic medium with omissions.....	Casein Hydrolyzate	24	0.74
Basic medium with omissions.....	Casein Hydrolyzate	192	1.05
Basic medium with omissions.....	Tryptophane plus Casein Hydrolyzate	0	0.00
Basic medium with omissions.....	Tryptophane plus Casein Hydrolyzate	24	0.51
Basic medium with omissions.....	Tryptophane plus Casein Hydrolyzate	192	1.11
Basic synthetic medium.....	None	192	5.72§

* All media inoculated with 0.1 ml. of suspension of Lactobacillus strain 333.
† See reference (3).
‡ Control for growth of organism.
§ Control for inhibition by incubated saliva.

The data of Table II show that saliva incubated with added glucose did not function as a complete replacement for the casein hydrolyzate of the synthetic medium, but did substitute satisfactorily for tryptophane. This observation may be interpreted as an indication (a) that some of the amino acids contained in the casein hydrolyzate were not liberated as readily during the incubation of saliva with sugar as was tryptophane, or (b) that the quantity of the other amino acids available in the incubated saliva was not sufficient for the needs of the growing organism. That the latter alternative may be the correct one is supported by the observation that the 3 ml. of incubated (with glucose) saliva added to the test medium contained only 0.35 mg. of free amino acid nitrogen whereas the casein hydrolyzate addition supplied 3.7 mg. of amino acid nitrogen to the medium.

SUMMARY

1. Human saliva incubated with glucose at 37.5° C. for 24 or more hours may be substituted for the added tryptophane in a synthetic medium which promotes the production of acid by *Lactobacillus acidophilus*.

2. Saliva incubated without added glucose caused only a minimal increase in the production of acid.

3. When saliva incubated with glucose was substituted for casein hydrolyzate in the synthetic medium acid production occurred but the quantity was only about one-fifth that obtained in the presence of the casein hydrolyzate.

The author wishes to express his appreciation to Dr. Harry C. Trimble, Department of Biological Chemistry, Harvard Medical School, for his valuable criticism and assistance in the preparation of this paper.

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CHANGES OF "CARBOXYL-CO_2" AND OF AMMONIA PLUS-UREA NITROGEN IN SALIVA INCUBATED WITH GLUCOSE[1]

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In 1911 Ruhemann (1) observed that ninhydrin (triketohydrindene hydrate) reacted with amino acids to produce carbon dioxide, ammonia and aldehydes. Recently Van Slyke and Dillon (2) and Van Slyke, Dillon, MacFadyen and and Hamilton (3) have reported that a-amino acids, when boiled in water with an excess of the reagent, evolve the CO_2 of their carboxyl groups quantitatively in a few minutes. Van Slyke, Dillon, MacFadyen and Hamilton have shown "that the reaction is specific for *free* amino acids in that it requires the presence, in the free, unconjugated state, of both carboxyl and the neighboring NH_2 or the NH—CH_2 group." They have stated that the ninhydrin-CO_2 reaction serves to differentiate free amino acids from peptides more sharply than is possible by any of the methods previously used for measurement of free amino or free carboxyl groups independently of the presence of the other.

Since the CO_2 evolved from free amino acids by the ninhydrin reaction can be measured rapidly and quantitatively in the manometric apparatus of Van Slyke and Neill (4) we have used the ninhydrin reaction to study the extent to which amino acids may be set free by the process of proteolysis in human saliva incubated at body temperature under several different conditions. These data were sought because observations recorded in the preceding paper of this series had shown that saliva incubated with glucose was capable of completely replacing the amino acid tryptophane and partially replacing casein hydrolyzate in synthetic media which support acid production by *Lactobacillus acidophilus*. It was hoped that the evidence resting upon a bacteriological basis for the occurrence of proteolysis in saliva at body temperature could be supported by experiments of a purely chemical nature. This expectation has been realized and this present paper is a record of the data obtained.

At the same time observations upon changes in ammonia-plus-urea nitrogen content of incubating saliva have been made. This was done in order to compare the effect of our experimental conditions upon the process of deaminization.

EXPERIMENTAL

Saliva collection and treatment.—Specimens of saliva for these experiments were collected and treated in the manner described in the preceding paper (12).

Chemical methods.—Samples of saliva were analyzed for free amino acids by the gasometric method of Van Slyke, Dillon, MacFadyen and Hamilton (3). Ammonia-plus-urea nitrogen was determined by the hypobromite method of Van

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Slyke (5). Measurements of pH were made with the glass electrode of a Coleman pH electrometer. Ammonia nitrogen was measured by the micro aeration method of Van Slyke and Cullen (6). Urea nitrogen was determined with the aid of urease by the method of Van Slyke and Cullen (6). Amino nitrogen was measured in a few experiments by the nitrous acid method of Van Slyke (7).

RESULTS

The data in Table I are typical of those obtained in four experiments in which saliva as obtained from human mouths was incubated at 37.5 C. Under this condition the quantity of ninhydrin-releasable CO_2 or the "carboxyl-CO_2"[2] of the incubated saliva, which was 1.52 mg. per 100 ml. at the beginning, declined by approximately one-third during the first 24 hours. Then it remained at approximately the same level for the next 48 hours when another drop occurred. At the end of 192 hours the level existing during the 24 to 72 hour interval was again approached. Simultaneously the ammonia-plus-urea nitrogen increased. In

TABLE I

Changes in "carboxyl-CO_2" and in ammonia (plus urea) nitrogen of incubating saliva

PERIOD OF INCUBATION	"CARBOXYL-CO_2" PRESENT	AMMONIA (PLUS UREA) NITROGEN	pH
	mg. per cent	*mg. per cent*	
0	1.52	20.9	8.25
24	1.19	42.1	7.26
48	1.08	46.0	7.36
72	1.02	42.9	7.50
120	0.42	45.9	7.47
144	0.67	45.9	
192	0.96	45.9	7.7

the first 24 hours of incubation the quantity of these substances present approximately doubled. There was an additional slight increase during the next day after which the quantity remained substantially the same until the end of the incubation period. In this experiment the pH declined slightly and then fluctuated slightly around pH 7.5 during the remainder of the experiment. In other experiments beginning at a less alkaline pH the initial decrease did not occur.

The data of Table II are taken from an experiment in which an autoclaved solution of glucose sufficient to produce a concentration of the sugar of about 1% was added to saliva before the incubation began. With this sugar present the quantity of ninhydrin-released or "carboxyl-CO_2" increased decisively during the first 24 hours. Thereafter it continued to rise until the incubation had been carried on for approximately 120 hours when the increase was more than 150% of the initial value. In another experiment the amino nitrogen of incubating saliva was measured by the nitrous acid method of Van Slyke (7). With saliva only, the quantity of nitrogen released by the action of nitrous acid remained approximately the same as the incubation progressed. In another test made

[2] Terminology of Van Slyke, Dillon, MacFadyen, and Hamilton (3).

simultaneously using saliva to which glucose was added the nitrogen released by the nitrous acid increased by more than 100% as the incubation progressed. Thus these experiments with the 2 types of reagents capable of acting upon different parts of amino acids show that free carboxyl groups and primary amino groups both increase as incubation progressed in the presence of glucose. Since the increases in quantity of the 2 groups were similar there is reason for believing that they were arising from the same source—the amino acids being set free by proteolysis in the incubating solution.

In the experiment shown in Table II the ammonia-plus-urea nitrogen remained at approximately the same level throughout the incubation period, while the pH decreased considerably. In other experiments of this series increases in the ammonia-plus-urea nitrogen content were observed, the largest however, being only about 15%. In contrast the increase in ammonia-plus-urea nitrogen when glucose was not added to saliva was 200–300%. Some years ago Kendall and

TABLE II

Changes in "carboxyl-CO_2" and in ammonia (plus urea) nitrogen of saliva incubated with added glucose

PERIOD OF INCUBATION	"CARBOXYL-CO_2" PRESENT	AMMONIA (PLUS UREA) NITROGEN	pH
	mg. per cent	*mg. per cent*	
0	1.52	20.9	8.25
24	2.63	21.5	4.15
48	3.11	22.4	3.90
72	3.83	22.2	3.82
120	4.07	22.6	3.68
144	4.08	22.6	
192	4.18	18.5	3.55

Farmer (8) observed that bacteria of a number of different types growing in cultures of various compositions produced more ammonia in the absence of, than in the presence of, glucose. Our findings with respect to glucose are of a similar sort. Waksman and Lomanitz (9) later showed that the increased production of ammonia nitrogen in the presence of glucose was greater than could be accounted for by the increased production of cell nitrogen. Stephenson and Gale (10) have demonstrated that glucose has no effect upon the metabolism of *E. coli* once the cell has been formed but the action must be upon the formation of the bacterial enzymes during the growth of the organism.

In control experiments it was demonstrated that increasing the urea nitrogen of specimens of saliva to 3 times the initial value produced no significant increase in the quantity of "carboxyl-CO_2" obtained. In this we are in accord with the observation of Van Slyke, Dillon, MacFayden and Hamilton (3). They found that urea in the amounts present in blood, which is several times the concentration existing in our experiments, did not interfere when amino acids were to be measured by the ninhydrin-CO_2 method. In other control experiments in which saliva was incubated alone and also with glucose for 48 hours the measurements

of the urea nitrogen and ammonia nitrogen were made separately. In these tests the urea nitrogen remained substantially the same throughout the experiment. Therefore all changes of ammonia-plus-urea nitrogen in Table I and II are to be taken as indications of changes in the ammonia content primarily.

Together the data of Table I and II indicate that the presence of glucose in incubating saliva promotes the liberation of amino acids (proteolysis) from substances which contain them in a combined form while deaminization reactions are very definitely decreased in rate if they were not actually halted. On the other hand when saliva is incubated without added glucose deaminization reactions are favored.

Since deaminization reactions can set free only 1 mole of ammonia from 1 mole of most of the amino acids and since ninhydrin releases only 1 mole of CO_2 from 1 mole of amino acids, other than aspartic (3), it is possible to use the data of Table I as indications of the source of the ammonia. In that table it is shown that the increases of ammonia-plus-urea nitrogen are a number of times greater than the available "carboxyl-CO_2" (liberated only from substances having a-amino as well as carboxyl groups free) originally present. Therefore it is evident that the increase of the ammonia-plus-urea nitrogen observed must have had its eventual source in the compounds other than free amino acids originally present. The data do not provide evidence that proteolysis had actually stopped during the incubation of saliva. The net decline in the "carboxyl-CO_2" during the experiment could have occurred if the rate of conversion of amino acids to ammonia by deaminization was slightly greater than the rate of the liberation of amino acids by proteolysis.

The data obtained in this investigation indicate that the quantity of free amino acids available in specimens of human saliva is quite definitely increased when the secretion is maintained at body temperature in the presence of glucose. These data of purely chemical nature support our previously reported findings that saliva is capable of replacing amino acids of a synthetic medium in which *Lactobacillus acidophilus* will produce acid only when the saliva has been hydrolyzed by acid or alkali (11) or when it has been incubated with glucose (12).

SUMMARY

1. The quantity of CO_2 liberated from human incubated saliva by the action of ninhydrin (designated as "carboxyl-CO_2") is increased approximately 100% when glucose is added.

2. The increase in "carboxyl-CO_2" provides evidence of a chemical nature for the liberation of additional amino acids in saliva maintained in contact with glucose at body temperature. This finding points toward free amino acids as the constituents of saliva incubated with glucose which enable saliva so treated to act as a substitute for tryptophane and to a lesser extent for other amino acids of a synthetic bacteriological medium.

3. The quantity of ammonia-plus-urea nitrogen increased from 200–400% when human saliva was incubated by itself for 24–192 hours. When glucose was added to the saliva the increases of ammonia-plus-urea nitrogen observed were

only in the order of about 15%. In each circumstance the increase was almost entirely in the ammonia fraction. The results are interpreted as evidence that the presence of glucose in saliva favors the process of proteolysis over that of deamination.

The author wishes to express his appreciation to Dr. Harry C. Trimble of the Department of Biological Chemistry, Harvard Medical School, for valuable criticisms and assistance in the preparation of this paper.

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ARGININE-ARGINASE RELATIONSHIP IN REGENERATION, REPAIR AND DEVELOPMENT OF NEOPLASMS

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In a series of experiments upon tissue cultures arginine and various substances containing arginine were incorporated in the media. These substances were also introduced into the cellular elements by the use of the micromanipulator. The results and reactions observed from these procedures seemed to warrant further investigation. Consequently an extensively organized series of experiments have been instigated and are now being carried on concerning the role of arginine and the enzyme arginase in the various processes of regeneration, repair, and in the development of neoplasms. The experimental evidence obtained will be briefly reported and discussed.

RESUME OF EXPERIMENTAL EVIDENCE

Tissue Culture

Connective tissue from the mesenteries, along with sections of parenchymal tissues of guinea pig, were incubated and virile colonies selected for this use. Incorporated in the media was arginine in various forms including guinea pig sperm and spermatic fluid, the protomine of which has an 87.4% arginine content. A concurrent experiment included the use of thyroxin in the media. It was observed that those cells under the influence of the arginine-containing media showed excellent growth with a preponderance of cells exhibiting mitosis. The controls did not show the mitotic figures. Those cultures under the influence of thyroxin exhibited larger cells and comparatively few mitotic figures. Incorporation of spermatic substance into the cultures resulted in growth comparable to that in cultures with pure arginine.

By the use of the micromanipulator both arginine and sperm were introduced into the nuclear substance. These cells became smaller than those not so treated, but assumed a mitotic nature, growing and multiplying independently (10).

The stimulative effect of thyroxin was readily observed as was that of arginine, the latter being estimated to be approximately twelve times that of the thyroxin, based upon cellular numbers. The areas of new cells resulting from thyroxin stimulation were considerably larger.

Animal Experimentation

Sixty guinea pigs were used, 30 of which were used as controls. Operative

[1] Received for publication August 20, 1946.

procedures, injections, and implants in all of the various types of tissues, with controls corresponding to these procedures maintained, gave ample evidence that arginine would and did produce local growths and nodules with many mitotic figures in the biopsies. Introduction of spermatic substances in ulcerous and traumatically abraided areas produced nodular growths around the periphery of these areas.

A new series of experiments to investigate the effect of both arginine and its ferment arginase with the mouse as the animal of choice have been under way for the past 2 years. Methods are being devised for obtaining arginase in varying degrees of purity for local application and injection (12). Indications are that purity is not of paramount importance, if one of the divalent activators of the enzyme is present. It has been observed that arginine is definitely stimulative to tissue growth, while its ferment has the opposite effect.

In one experiment, 20 white mice and 20 controls were used, and solutions of various strengths of arginase were injected subcutaneously and intraperitoneally. The systemic effect was observable in a corresponding weight curve. This curve indicated that the effect of the enzyme was quantitative. The local effect was such that dissolution of tissue around the initial lesion was also quantitatively relational to the strength and quantity of the enzyme used.

Just what bearing the guanidine content of the amino-acid "arginine" has upon the observations is now under investigation. The comparative effects of arginine, arginase and guanidine upon embryonic tissues are also being studied.

DISCUSSION

It is somewhat early to attempt a complete discussion of the results of these combined and inter-related experiments. However, evidence is accumulating (1, 2) and reports are now appearing in the literature which seem to substantiate these findings (8, 9) to the point where an hypothesis can well be formulated.

Arginine-arginase plays a definite role in metabolism of local repair and regeneration. This can well be regarded as one of the positive results of the experiments. The physiology of this seems to indicate the following explanation:

Arginine stimulates the liver to produce arginase which is then taken up and is present in the intercellular substances of the tissues along with its substrate. A condition which calls for regeneration or repair releases from the cells the inhibitors of arginase, leaving the arginine free to act directly upon the cells involved. The result is a local regeneration or cellular multiplication. As continuity is reestablished, the arginine-arginase bond is again resumed with subsequent inactivity of the arginine (3). This has been shown experimentally to be a truly quantitative reaction. Under conditions of trauma, freed arginine may form a number of bonds with the nucleic acid of an affected cell (5, 6). Continued traumatic stimulation, or that of morbid physiology is the stimulus that activates this unit toward an autonomous growth process (11). In the event of hepatic insufficiency, arginase may not be produced adequately (4), if at all; then arginine has a free rein in its activities upon cellular substances and organization.

Adrenalectomy and hypophysectomy have the effect of decreasing liver arginase. Administration of pituitary hormone directly increases liver arginase (7, 8). This apparently places the arginine-arginase action under the influence of the endocrine system.

SUMMARY

1. Arginine plays an important role in the development of the anlage of embryonic tissues. (87.4% of protamine of sperm is arginine.)

2. There is an arginine-nucleic acid relationship that may remain latent until stimulated into activity, resulting in autonomous growth or regenerative process.

3. Arginase, produced primarily in the liver, exists in the tissues in combination with arginine. The normal metabolic anabolism is regulated by this combine, by altering the balance between the enzyme and its substrate.

4. Arginine, combining with nuclear material of an injured cell supplies the same nuclear conditions that cause the autonomous growth of the fertilized ovum.

5. Hepatic insufficiency prevents the inhibition of arginine stimulation.

6. Arginase-arginine relationship is under the influence of the hormonal system of the body.

7. Arginine, necessary for local regenerative processes, must be furnished, at least in part, by food intake; therefore, nutritional disturbances may also be considered a source of enzyme-substrate unbalance.

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THE SUPPORTING TISSUES OF THE TOOTH IN ACUTE SECONDARY AGRANULOCYTOSIS (ARSPHENAMIN NEUTROPENIA)[1]

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Of all the lesions produced in the mucosa of the oral cavity and the pharynx by blood dyscrasias, the most marked and extended are those associated with so-called "agranulocytosis." The gingiva, tonsils and peritonsillar areas bear most damage. These tissues often appear involved even before other signs of the disease become obvious. Since the disorder shows, among other features, a decrease rather than a total absence of polymorphonuclear cells the term "acute neutropenia" has been suggested as more appropriate.

While the oral manifestations in various types of leukemias were microscopically studied in the past, it was not until 1933 that the first microscopic examination of the gingiva and jaw lesions in a case of arsphenamin "agranulocytosis" which came to autopsy was reported by the author. This early study dealt mainly with the description of the changes of the alveolar bone. This discussion attempts to answer the question why the agranulocytotic lesions appear mostly in the marginal gingivae before they proceed further. The cellular reaction in the gingiva and the changes in the jaw bones will be described also.

More recent elaborate studies by Fitz-Hugh and Krumbhaar, Jackson and Parker, and especially, Custer emphasize the difference between idiopathic agranulocytosis and secondary agranulocytosis, which is the result of a particular hematologic sensitivity to certain drugs or microorganisms. Though the clinical symptoms of the 2 types are alike and include fever, chills, and necrotizing process in the mucosa of the mouth and throat without anemia or thrombocytopenia, Custer maintained that the bone marrow in secondary neutropenia differs from that in idiopathic agranulocytosis. Custer examined regenerating marrow and found that in the former "granular forms in the neutrophil series predominate" while the latter is characterized by the complete failure of proliferating myeloblasts to mature into granular neutrophils. Agreeing with Fitz-Hugh and Krumbhaar, Custer believed that the absence of an "intrinsic factor" prevents the maturation. The nature of this "intrinsic factor" is unknown. Custer's findings and conclusions were confirmed by the clear-cut study of Darling, Parker and Jackson. The clinical picture of the "idiopathic" and "secondary" neutropenia is supplemented by distinct findings in the bone marrow such as an increased number of myeloblasts, lymphocytes, plasma cells and marked formation of red blood cells.

[1] Presented at the 24th General Meeting of the International Association for Dental Research, March, 1946 (*J. D. Res.* **24**: 192, 1945). Received for publication August 5, 1946.

Although the authors agree on the above mentioned clinical and hematologic features, no uniformity of opinion exists as to the commoness of bleeding. Those who denied hemorrhagic manifestations in "agranulocytosis" (Jackson and Parker, Custer) considered hemorrhage "strong evidence that one is dealing with some form of acute leukemia" (Jackson and Parker). Jackson and Parker contended that "acute leukemia in the aleukemic phase very closely simulates agranulocytosis and the two diseases are not infrequently mistaken one for the other. In fact, for the present, the relationship of the two conditions must be regarded as problematical."

Reznikoff recognized bleeding only "as a result of vascular involvement by a necrotic lesion." However, Roberts and Kracke pointed out that it is "rare to see a case (of agranulocytosis) in which there is not some manifestation of the hemorrhagic trend," and "no hemorrhages may be seen during life and yet necropsy may reveal many." Kracke and Garver also reported hemorrhages in agranulocytosis. Recently Rosenbach studied the agranulocytotic changes of the gastrointestinal mucosa and described congested vessels, edema and hemorrhages.

Though bleedings do not, as some authors state, fit the picture of agranulocytosis, 2 of the 3 cases studied by the author showed microscopically, but not grossly, hemorrhages in the gingiva and the superficial bone marrow of the jaws.

OBSERVATIONS

The clinical and hematologic findings ascertained the diagnosis of arsphenamin agranulocytosis. The 2 females, 24 and 28 years of age, succumbed within 4 and 10 days while the third individual, a male of 26 years died after about 2 months. All 3 had received neoarsphenamin for antisyphilitic treatment and each developed gangrenous gingivitis and stomatitis. These oral lesions were moderate in the 2 cases of short duration but very extensive in the case of 2 months duration.

The number of the white cells in the 3 cases ranged between 2000 and 2800 and the differential counts showed 23–30% polymorphonuclear leukocytes, 60–69% lymphocytes and 8–10% monocytes. While neither of the 2 females revealed an anemia, the male did show it. The platelet counts were normal. Spleen and lymph nodes were not enlarged.

The examination of the sternal bone marrow revealed a remarkable difference between the case of 2 months duration and the 2 cases which went rapidly downhill. The former showed plasma cells, lymphocytes and many normoblasts while the 2 latter presented a marrow with few polymorphonuclear cells, many myeloblasts, a moderate number of plasma cells, few lymphocytes and normal erythroblastic activity.

At autopsy the jaws of the 2 females and the male were removed for histologic examination. Since the histo-pathologic changes observed in the jaws of the 2 females are alike, they will be discussed together:

The microscopic examination of the upper teeth in connection with the jaws

revealed unaltered fatty marrow in the regions farthest from the teeth. The marrow close to the teeth showed bleeding, the intensity of which increased toward the alveolar crest while the fat cells decreased in number (*fig. 1*). Congested vessels, edema and an increasing number of plasma cells, myeloblasts, normoblasts, and lymphocytes were observed in these hemorrhagic areas. The marrow closer to the crest and the periodontal membrane became more fibrous and proliferation of the capillary endothelium was noticeable.

Distant from the teeth, the surface of the bone trabeculae of the cortex and those of the spongy part did not show any changes. However, in accordance

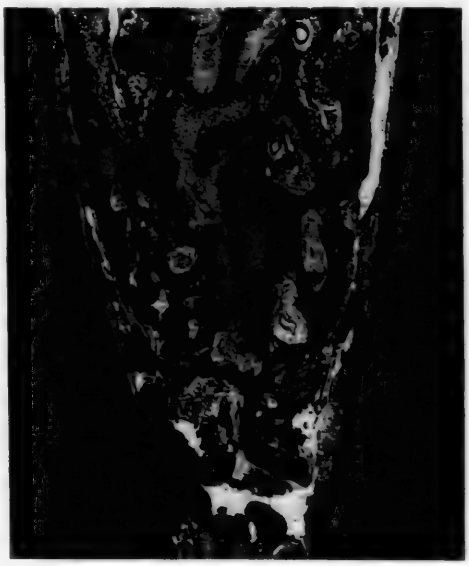

Fig. 1. Frontal section through the central maxillary incisors in acute arsphenamin agranulocytosis, showing areas of both roots with the interdental alveolar bone. Extensive bleeding in the periodontal membranes and bone marrow. Osteoclastic resorption of bone trabeculae.

with the changes of the marrow described above, the trabeculae adjacent to the teeth were osteoclastically resorbed. Deep Howship's lacunae with or without osteoclasts lined the bone which appeared thinned and even destroyed to such an extent that broad communications between periodontal membrane and marrow spaces of the supporting bone were noted. Some sections showed the disappearance of the entire partially necrotic labial part of the alveolar process. Many osteoclasts were detached from the lacunae by hemorrhages. The marrow of the alveolar crest was necrotic.

The mandibular first premolar of one of the females showed a large chronic periapical lesion composed of granulation tissue which was traversed by strands of epithelial cells. A cavity in the center lined by flat epithelium contained

chiefly blood and very little pus. Doubtless, this was an abscess the pus of which had been mostly replaced by red blood cells. The bloody material originated from the extensive hemorrhages present in the surrounding tissue. The outstanding feature of this lesion was the almost total absence of polymorphnuclear leucocytes and lymphocytes but the presence of an overwhelming number of plasma cells and myeloblasts.

A similar cytologic feature was found in the preserved apical rest of the pulp of a maxillary canine the root canal of which contained a guttapercha filling. A

Fig. 2. Photomicrograph showing labial marginal gingiva and oral epithelium of a maxillary canine in acute arsphenamin agranulocytosis. Hemorrhages and necrosis advanced gradually from the gingival sulcus into the oral epithelium which is fairly well preserved.

great number of lymphocytes and plasma cells crowded this area and this was also observed in the thickened apical part of the periodontal membrane.

The periodontal membrane of the maxillary and mandibular anterior teeth and premolars was occupied by hemorrhages. The Sharpey's bundles were destroyed except for the apical region where the fibers were well preserved though free blood appeared collected between them. This accumulation of blood continued into the pulp. Here and there small fragments of necrotic bone were displaced into the periodontal hemorrhages. The cementum of the teeth in these 2 acute cases was not altered.

The pulps of some teeth showed reticular atrophy with hemorrhages. Their odontoblasts exhibited a hydropic degeneration. The pulps of other teeth were unchanged in spite of the remarkable findings in the tooth-supporting structures.

The changes of the vestibular and palatal mucosa of the alveolar processes were remarkable. Extravasated blood extended along the outer surface of the alveolar processes either directly adjacent to the osteoclastically resorbed bone or between the periosteum and subepithelial tissue. Very few polymorphnuclear

Fig. 3. Photomicrograph showing labial marginal gingiva and oral epithelium of a maxillary canine in acute arsphenamin agranulocytosis. Intensive necrosis of the gingiva next to the gingival sulcus decreasing towards oral epithelium.

cells were present while plasma cells, macrophages and lymphocytes were abundant. Neither the epithelium nor the subepithelial tissue exhibited signs of necrosis. However, the marginal parts of the gingiva revealed complete necrosis with ulcer formation. This degenerative change, characterized by the total loss of any structure, was very obvious next to the gingival sulcus. It was in this area that the necrosis started. From here it progressed toward the epithelium of the surface of the gingiva and involved it finally. Numerous plasma cells and lymphocytes were between the necrotic tissue and the areas not yet affected.

Gradually, the necrosis advanced into the periodontal membrane and occasionally also into the vestibular mucosa.

The microscopic findings of the jaws of the male who succumbed after more than 2 months duration to the disease differed essentially from those described

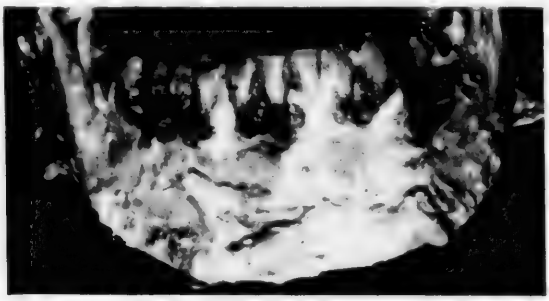

Fig. 4. Gangrenous lesions of the gingiva in arsphenamin agranulocytosis of about 2 months duration.

Fig. 5

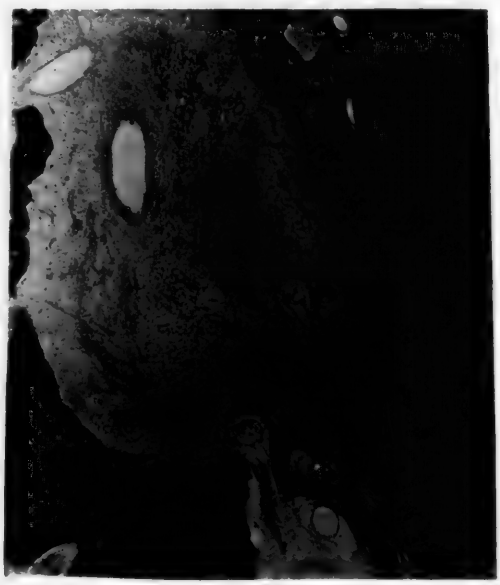

Fig. 6

Fig. 5. Mesio-distal section through the first mandibular incisors of the specimen shown in *fig. 4*. Note osteoporosis, destruction of alveolar socket, new bone trabeculae around the apical ends of the teeth, necrosis of gingiva.

Fig. 6. Higher magnification of *fig. 5* showing resorption of cementum and irregular formation of new bone. Fibrous bone marrow with many capillaries and numerous normoblasts.

above (*fig. 4*). The fatty marrow in portions of the mandible and maxilla away from the teeth contained dilated capillaries which were encircled by broad layers of collagenous materials. Edema and a reticular fibrous tissue separated the fat cells from one another. Many normoblasts and few lymphocytes and

myeloblasts were scattered throughout the marrow. The vessels did not contain any granulocytes and plasma cells were not noted in the tissue (*fig. 5*).

The spongy bone of the jaws showed an advanced osteoporosis. Only few bone trabeculae were in moderate apposition. The surfaces of most of them were void of any cells thus indicating a quiescent stage. The nearer to the apex of the teeth the more fibrous the marrow appeared. Some of the apical bone trabeculae showed osteoclastic resorption. The most remarkable findings in this area were newly formed thin bone trabeculae which as a kind of reparative formation partly replaced the bone of the alveolar crest and extended in an irregular fashion into the periodontal space. Newly deposited bone was also observed in the bone marrow spaces adjacent to the alveolar crest.

The remaining extremely widened periodontal membranes were transformed into a very dense fibrous tissue the fibers of which ran parallel to the surface of the roots. There was bleeding as a result of mechanically severed vessels.

The cementum showed deep grooves from resorption all along the root. Some of these Howship's lacunae in the apical third contained cementoclasts, while those in the marginal part were repaired by deposition of bone substance (*fig. 6*).

The fibrous marrow close to the crest communicated by broad openings with the subepithelial gingival tissue. Dilated vessels and a large number of normoblasts were scattered throughout. Very few lymphocytes and no polymorphonuclear leucocytes were present.

The epithelial lining of the marginal gingiva and the interdental papillae were necrotic. The necrosis also involved the subepithelial tissue and the normoblasts therein. There was definite evidence that the necrosis progressed from the epithelial attachment outward and in the apical direction thus involving neighboring parts of the gingiva, the vestibular mucosa and the alveolar process. This was particularly obvious in the mandibular premolar and molar region where large areas of the alveolar process were exposed and necrotic.

DISCUSSION

The 2 very acute cases described showed gangrenous defects of the gingiva associated with microscopically established bleedings limited to the oral mucosa. The ages of the patients, the acuteness and rapid death , and the diffuse bleedings in the oral mucosa would lend one to think of cases of primary aplastic anemia or acute aleukemia. However, since the disease followed the intravenous administration of arsphenamin, the blood-platelet count remaining high and the bone marrow revealing a remarkably decreased number of mature granulocytes with numerous myeloblasts, normoblasts, plasma cells and lymphocytes, study clearly indicated arsphenamin agranulocytosis.

The third reported case, of 2 months duration, exhibited extensive gangrenous defects of the gingiva and oral mucosa, but only remains of old hemorrhages were observed microscopically in the gingiva. The bone marrow was almost "empty" but contained many normoblasts.

It appears from the microscopic examination of the jaws of these 3 lethal cases of secondary granulocytosis that the necrotic process of the gingiva commences

in the tissue bordering the gingival sulcus. It is from here that the necrosis spreads toward the gingival epithelium, into the periodontal membrane and the marrow of the alveolar process as well, thus destroying the supporting tissue of the teeth. The start of the necrosis can be intelligently explained with the presence of the bacterial flora in deepened gingival crevices. The remarkably reduced number of phagocytosing granular leukocytes or their absence permits the microorganisms, particularly the anaeorobes lodging in the crevices, to act upon the adjacent tissue and bring about gangrenous lesions.

The gangrenous process involves the periodontal membrane and causes in the very acute cases a quick destruction of the alveolar process, while in the protracted cases limited reparatory processes of the alveolar bone took place.

Ulcers in agranulocytosis are found also in the tonsils, pharynx, in the mucosa of the gastrointestinal tract, and the vagina. These organs are lined by a mucosa the numerous foldings of which harbor microorganisms which produce in necrotic lesions identical to the ulcers in the gingiva.

CONCLUSIONS

1. The necrotic lesions of the gingiva in secondary agranulocytosis start in the tissue next to the gingival sulcus. The microorganisms, including many anaerobes lodging in the deepened gingival sulcus, act quickly upon the gingival tissue because of the lack of phagocytotic granulocytes.

2. The necrosis spreads from here toward the surface of the gingiva and into the periodontal membrane and the marrow of the alveolar process thereby destroying the tooth supporting tissue.

3. The agranulocytotic ulcers in the tonsils, the mucosa of the pharynx, intestine and other organs have identical origin. The necrosis commences in the crypts and folds of these structures which harbor microorganisms.

4. The subepithelial tissue and the marrow of the alveolar process adjacent to and distant from the necrotic lesions of the rapidly fatal cases contain numerous plasma cells, lymphocytes, but few normoblasts and polymorphnuclear leucocytes. The same areas in a case of 2 months duration exhibit only numerous normoblasts and few lymphocytes.

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RACHITIC CHANGES OF THE MANDIBULAR CONDYLE OF THE RAT

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The rachitic changes in the epiphyseal plate and the metaphysis of tubular bones are well known (2). As a result of vitamin D deficiency, the hyaline cartilage of the epiphyseal plate does not calcify whereas proliferation of cartilage progresses for a longer period. The uncalcified cartilage is resistant to resorption, preventing replacement by bone at the metaphyseal border. As a consequence of the progressing proliferation and the lack of resorption, the epiphyseal plate increases in thickness to 3 to 4 times its physiological width. The bone formed during the rachitic regime remains likewise uncalcified and persists as osteoid tissue which is also resistant to resorption (3). Thus, the normal reconstruction and modeling resorption of the growing bone ceases, leading to increasing density of the spongiosa and to a clublike thickening of the metaphysis.

Rachitic changes of the mandibular condyle have not been described. It is known that the mandible grows at the condyle in a manner similar to the growth of tubular bones at the epiphyseal plate and the articular cartilage (4). It was therefore of interest to investigate the condyle of rachitic animals and to ascertain whether the changes of the mandible are comparable to those seen in tubular bones.

MATERIAL AND TECHNIC

Fourteen white rats were kept on rachitogenic diet (Steenbock and Black (5)) for 7 weeks after weaning. The animals were then sacrificed, the heads halved and fixed in Zenker-formalin solution (Maximow). After decalcification, a block was cut out which contained the temporomandibular joint. This was done by making 2 frontal cuts, one immediately behind the eye, the other just in front of the ear. Frontal sections were made and stained with hematoxylin-eosin. Control material from litter mates was obtained in the same manner. Frontal sections through the joint are advisable because of the shape of the condyle which is elongated in antero-posterior direction and narrow in medio-lateral direction. To obtain comparable sections, the Gasserian ganglion and the foramen ovale are used as landmarks.

FINDINGS

In a frontal section the normal condyle presents the following picture (*fig. 1*). The bone of the condyle is covered by a crescent shaped hyaline cartilage, which

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itself is lined toward the cavity of the joint by a layer of dense connective tissue. The cartilage shows 3 zones: in the peripheral zone, the chondrocytes are small, separated by wide bars of hyaline ground substance; in the middle zone, the cytoplasm of the chondrocytes has increased in size and the cells are arranged in short columns; in the inner zone, the chondrocytes show signs of degeneration and their capsules are opened by the cells of the proliferating connective tissue. The

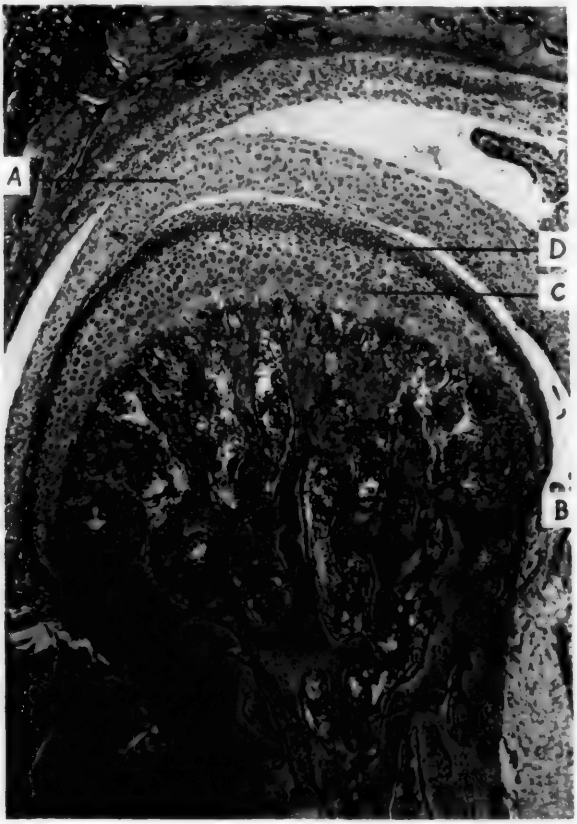

Fig. 1. Frontal section through the condyle of control rat 10 weeks of age, fed purina dog chow. A—Articular disc, B—Bone, C—Condyler cartilage, D—Fibrous covering of condyle. Mag. ×57.

connective tissue which covers the cartilage functions in the double role of articulating cushion and perichondrium. In the latter capacity, it is the mother tissue of the cartilage: its deepest layers show a gradual transition between connective tissue and cartilage, thus contributing to the appositional growth of the cartilage.

The cartilaginous convering of the bone reaches laterally and medially to the level of the widest part of the head. Below this level, the bone is constricted to the narrow neck of the mandible. The bone consists of spongy trabeculae which

end at right angles on the inner surface of the cartilage and suround relatively narrow marrow spaces. A cortical layer is inconspicuous in the region of the neck and gains considerable thickness only some distance below the condyle. The bone marrow is of the hemopoietic cellular type.

The rachitic condyle shows a characteristic disfiguration (*fig. 2*). The cartilage, crescent shaped in the normal animal, has grown to a thick plate almost

Fig. 2. Frontal section through the condyle of a rat 10 weeks of age kept for 7 weeks on rachitongenic diet. A—Articular disc, B—Osteoid tissue, C—Condylar cartilage, D—Fibrous covering of condyle. Mag. ×57.

square in the frontal section. The characteristic constriction at the neck has disappeared entirely. The thick spongy trabeculae of the bone consist of a core of calcified bone surrounded by thick layers of osteoid tissue. Peripherally there is a fairly thick compact layer which consists entirely of osteoid tissue. This layer extends somewhat over the cartilaginous part of the head. The marrow spaces in the region of the neck are severely narrowed; some are almost obliterated and contain mostly loose connective tissue instead of hemopoietic marrow.

DISCUSSION

The finding on the rachitic mandibular condyle are, in principle, identical with those of the rachitic epiphyseal or articular cartilages. They are the consequence of the inability of cartilage to calcify and the inability of the cells of the connective tissue to resorb the uncalcified cartilage. Thus, the proliferation of the cartilage goes on without its replacement by bone; hence the hyperplasia of the growth cartilage. Conversely these findings are proof for the function of the condylar cartilage as the most important growth center of the mandible. The identical findings in the condylar cartilage and in the epiphyseal and articular cartilages justifies the assumption that also in the condylar cartilage, the proliferation during rickets will not continue indefinitely. In other words, the assumption is justified that rickets will lead to a slackening and finally to a cessation of mandibular growth. This is also circumstantial evidence for an explanation of rachitic disturbances of the vertical eruption of teeth as the consequences of a primary disturbance of mandibular growth (6). Vertical tooth eruption depends on the vertical growth of the mandibular ramus by which the necessary space for tooth eruption is provided (1).

The findings on the bone of the mandible in experimental rickets are in agreement with the theory that increased density and transversal enlargement of bone in rickets is mainly caused by cessation of reconstructive and modeling resorption. This, in turn, is the consequence of the inability of the osteoid tissue to calcify.

SUMMARY

The effect of rachitogenic diet on the mandibular condyle of the white rat is, in principle, analogous to that observed in the articular and epiphyseal cartilages of tubular bones. The growth cartilage in the condyle is increased in thickness. Tlhe condyle itself is clubshaped. Both phenomena are the consequence of the lack of calcification of the cartilage and the osteoid tissue, and their consecutive resistance to resorption.

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COMPARATIVE ALVEOLAR PATHOLOGY IN SHEEP AND GOATS[1]

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Degenerative change of the jaw bone is one of the manifestations associated with old age. Although, infrequently, exceptions occur where the physical preservation of the jawbones remains unaltered and in a healthy condition until an advanced age, structural wasting and cellular degeneration predominate overwhelmingly. This process of change from youth to old age and from health to disease generally occurs among lower animals as in man. Although the onset of the wasting of the bone and the morphologic pathology differs among individuals, the portion of the bone affected in most instances, whether man or lower animal, begins with the alveolar process.

Alveolar degeneration, as it occurs predominantly in man and perhaps in most animals, excepting sheep, consists, of 2 chief types. These are: 1, atrophy or wasting of the cellular structure in the absence of pus, and 2, suppurative necrosis which destroys the walls and the septi of the tooth sockets. In either instance the breakdown of the alveolus begins at the alveolar crest, gradually reducing the periodontium either to the level of, or below the level of, the root ends. Also, there are instances of localized mechanical injury to the alveolar bone caused by incrustation of calcific deposit or continued packing of food between the teeth. The latter condition becomes more aggravated in the helpless animal, which is unable to remove such an obstruction. Although unlike collections of calculus which may occur at various periods of life of the individual from preadolescence through old age, packing of food between the teeth usually begins during middle or late adulthood. At that period some alteration of the alveolar support of the teeth frequently takes place, detachment of the periodontal membrane from the cementum occurs either partially or totally and the roots of the teeth begin to lose their firm hold in the alveolus. Under the impact of forced pressure during mastication teeth thus affected will yield to the force of the opposing teeth and the food. The resulting space is large enough for food particles to lodge and accumulate. Gradually, with the constantly added food creating separation and more space between the teeth, the shifting of the teeth forms larger pockets for a still greater amount of food to pack firmly thus depressing the gingiva and the affected bone. Especially in the herbivorous animals, the retained grass and twigs aggravated by continued pressure during mastication, produces channeling of the bone in the interspaces of most of the teeth posterior to the canines. Although packing of food results in extensive damage to the alveolar bone between the roots of the teeth of man, the actual destruction seldom proceeds beyond the root ends unless an abscess cavity in the jaw bone results from some other cause.

[1] Received for publication August 5, 1946.

In the sheep, particularly in the lower jaw, in addition to this damage to the bone an entirely different type was found.

The conditions discovered during this study were not anticipated. The purpose of the original inquiry was to make a comparative study of pathologic changes in the jaw bone of the human and the sheep. It is well known that nothing prevailed in the history of primitive man or lower mammal to indicate a tendency towards profound deterioration of the alveolar support of the teeth such as exists in civilized man. It appeared that the domestic sheep, long removed from the need of struggle for existence, requiring natural and human protection and wholly dependent upon restricted adaptation of environment and

Fig. 1. Mandibular sections from young to middle-adult sheep. At this stage of animal's life, bone is intact and roots of teeth are firmly invested in their sockets. Note unusually large mandibular canals.

food habits, ought to provide corollary information. The food habit that places a limited demand on the masticatory system, as is the case with the sheep, should reveal the functional effect on bone structure.

OBSERVATIONS

Thirty-six heads of freshly slaughtered sheep were used for this study. The animals came from farms within the states of Ohio, Kentucky and Indiana. In age these sheep ranged from 6 months to an estimated 12 to 14 years[2]. However, the middle and late adult was the objective of this study.

Normal conditions were found in young animals, the bodies of the mandible or maxilla, including the alveolar process of both, consisted of thick and dense bone, the mandibular canal was found to be unusually large, no doubt normal for sheep (*fig. 1*), no evidence of pathologic changes was present either in the jaws or al-

[2]This material was obtained from separate sources, and the work done in several stages.

veolar process, and the teeth were in normal arrangement with no grass fibre lodged between the teeth.

An entirely different picture revealed itself in the jaws of sheep estimated at 6 years of age or older. The tendency for packing of grass fibre appeared with greater frequency at and beyond this age period. Retained food, damage to alveolar bone and loose teeth were limited and confined to few teeth in the middle adult, but were present between practically all of the back teeth in the older animals. That old age, loose and lost teeth, and badly damaged alveolar support run a concomitant course could no longer be questioned even before the jaws of the older sheep were sectioned. The tendency for exfoliation of shaky teeth in the sheep does not occur as frequently as in the aged human, in only a few instances in the old animals was it noted that teeth were missing. This fact appeared as significant in view of the overwhelming frequency of loose teeth and the ever-present disease and hopeless condition of the alveolar support (*fig. 2*). Although wear of the teeth to an excessive degree in the older animals was often encountered, the wear was not always uniform. This was indicated by the uneven reduction of the crowns of the teeth in older animals. One concludes that chewing with some of the teeth was avoided, doubtless due to discomfort.

DISCUSSION

This limited study of the jaw bones of the sheep revealed degenerative age changes that have no counterparts in the process of senile changes of the human skeleton and, perhaps, not in other mammals. For example: The human mandible in old age loses its proportional dimensions which, is due primarily to the loss of the alveolar process as well as to further wasting of the mandibular body. A considerable proportion of the compact walls, as well as the medullary bone (except in extreme instances of atrophy), usually remains until later periods of life. Where extreme shrinkage does occur, it usually follows at the expense of the spongiosa and medullary bone. Invariably, however, the greatly reduced body of bone is transformed into a solid and dense remnant of the former mandible. The same process of change occurs in the maxilla.

An entirely different phenomenon occurs in the degenerative change in the sheep during old age. Again citing the mandible as example, it was found that no appreciable shrinkage of the alveolar process occurs in spite of the loss of alveolar bone and looseness of the teeth. Neither is there any evidence that there was an apparent reduction in the height or depth of the body of the mandible. Sections (macroscopic) of the jaws through the full length of the teeth from the youngest to the oldest has disclosed that while the compact bone in the young sheep exhibits what may be regarded as normal thick walls of the mandible, there is a gradual reduction in thickness within the body of the jaw at the turn from middle life towards old age.

The indications are that, first, there is a detachment of the periodontal membrane from the cementum. Due to the loss of function, no doubt, the membrane is slowly exfoliated leaving a space between root and cancellous bone (*fig. 3*). Cellular dissolution of the cancellous bone follows and soon permeates the compact inner walls of the mandible. At this stage, the compacta which previously

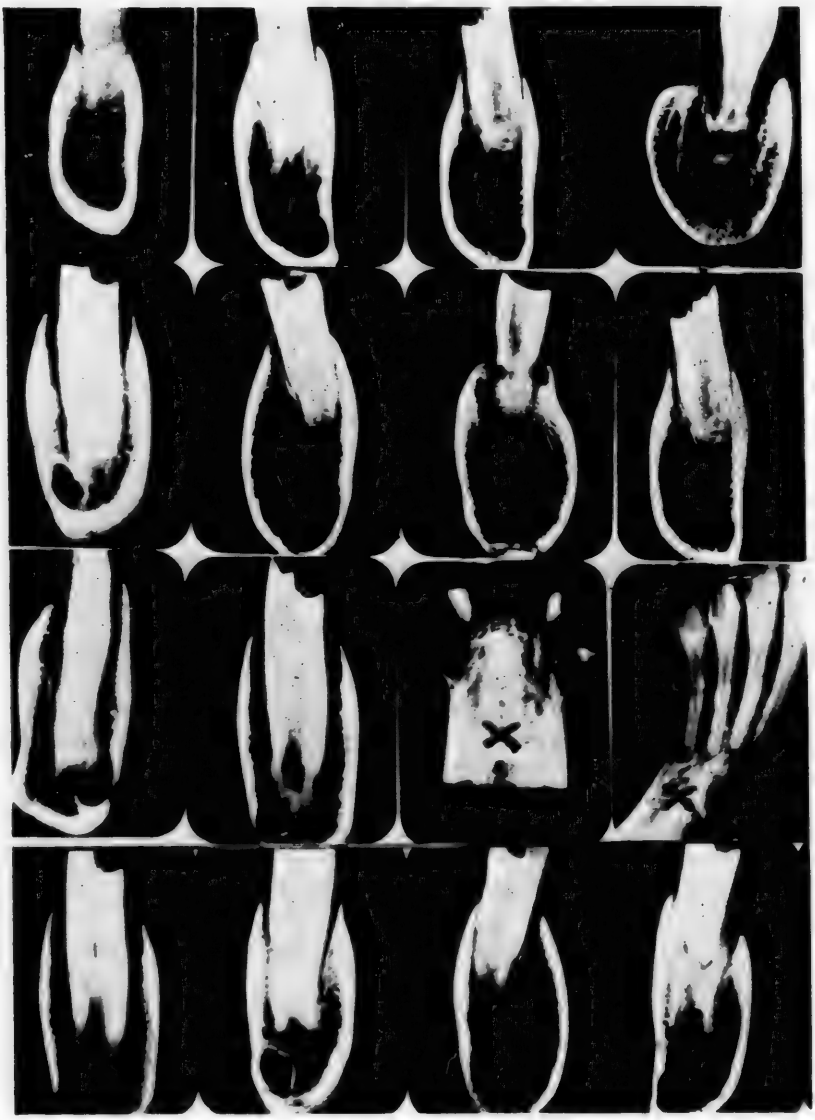

Fig. 2. Radiographs of mandibular sections. Note absence of medullary bone in most specimens. The process of change, however, from normal condition to disease is not universally similar, as it is revealed in each section. Lower anterior teeth in sheep, (X marked), have no bone sockets. Roots are firmly held in place by fibrous attachment which is tenaciously adherent to bone and roots. Gingiva also provides added support to teeth to which it is firmly attached. Teeth are not rigidly fixed to bone; they yield to pressure consistent with resiliency of tough portion of gingiva in that area. Despite lack of rigidity of roots, teeth continue to function and wear down until reduced to fragments, and these require effort to remove. In large mountain sheep, however, anterior teeth have regular socket support.

consisted of dense and homogenous bone is changed to a porous consistency. Further resorption continues as the smaller formed openings in the bone enlarge leaving thin septi in a sparse lace-work design. Ultimately this lace-work is resorbed, and, in many instances, large portions of the inner jaw are reduced to a thin outer shell (*fig. 4*).

Most characteristic in this entire process of change from normal to pathologic, is the replacement of resorbed bone by fat. Fat was found in the smaller interspaces in the sections from middle aged animals and hollow in the bony containers

Fig. 3. Mandibular sections. Top row: separation of roots from jaw bone, periodontal membrane still adhering to bone; unattached roots are covered with debris. Bottom row: sections still retaining firmly attached periodontal membrane. Bone had lost its homogenous consistence; it is soft, porous, and easily pierced with an instrument.

of the late senile specimens. Similar conditions were found in the maxillae, where resorbed bone was being replaced, continually, with fat. It may be postulated that functional disuse of the jaws and teeth in the old animal may in part be responsible for the degeneration of the bone. Coronal and sagittal sections through the palate and calvarium disclosed conditions similar to that in maxillary bone—sparse, spongy bone with interspaces filled with fat. On cutting, the bone throughout the skull was found to be surprisingly soft. The sinuses of the sheep head are extremely large and the ticks in the nasal chambers are extremely numerous.

Following the experience with the sheep, a further attempt was made for a corollary study on the goat. Difficulties were encountered, however. From the

Fig. 4. Top row: Sagittal sections of mandible through crown of sheep teeth. Note characteristic dissolution of the medullary bone which is followed by resorption of the compacta. This process of change from normal to disease is distinctly exhibited in middle row, particularly center- necrosis- and adjacent 2 sections- mere shells.

Bottom row: Maxillary sections through teeth and sinus. Cellular breakdown is similar to the mandible, even though, in appearance, the destruction of bone is not so profound; the empty spaces are obscured by an accumulation of fat.

outset, scarcity of material was the major obstacle. Only 3 adult heads could be obtained. These, too, were from freshly slaughtered animals, estimated ages

between 5 to 7 years. Equally disappointing was the result obtained from even this meager amount of material. It was soon discovered that cutting of the cranium with a bone band saw could be accomplished, but not through the crowns of the teeth. After several attempts at sectioning goats jaws through the tooth crowns, this had to be abandoned. Dulling and breaking of ordinary bone saw

Fig. 5. Portions of upper and lower jaws of goat: top, buccal; bottom: lingual surfaces. Bone is extremely dense and massive; alveolar crest of neither upper nor lower jaws was impaired; outer cusps of teeth are sharp, saw-shaped and appear highly mineralized

blades (the same used for other types of bone and human teeth) on goats' teeth precluded further interest in the goat. The following evidence, however, was obtained notwithstanding the difficulties of sectioning the specimens: 1, The bone of the entire skull, particularly the jaws, is massive, dense, and heavy; 2, the teeth appear highly mineralized; the cusps are sharp, with the worn surfaces of the enamel having a more distinct lustre than is observable in the teeth of the sheep or humans (*fig. 5*).

That rapid deterioration and disease of the jaw bones may be induced in the

goat resulting from a change in adapted diet is briefly mentioned here from a discussion on the subject by Knaggs. In discussing osteitis fibrosa in goats Knaggs[3] quoted W. G. Spencer's account of disease in goats kept for milking purposes in South London. The diet of these goats consisted of hay, corn, cooked vegetables and garbage—a diet that differed from goats fed on their natural foods such as hay and coarse upland grass. Knaggs described the disease that manifests itself by symmetrical swellings of the mandible, which gradually increase until the mouth cannot be closed and death results from starvation.

SUMMARY

Sheep jaws show changes with age that are far different from those in the jaws of man. The alveolar processes of sheep show no appreciable reduction in spite of looseness of the teeth and loss of supporting bone. Goat jaws appear to be extremely dense and heavy with highly mineralized teeth.

[3]Knaggs, R. Lawford, The Inflammatory and Toxic Diseases of Bone. (London, Bristol, John Wright and Sons Ltd.: (1926) p. 265.

WILLIAM J. GIES ENDOWMENT FUND, JOURNAL OF DENTAL RESEARCH[1]

Financial Statement as of March 11, 1946

Compiled by LEUMAN M. WAUGH, D.D.S., *Treasurer of Committee on Endowment*

This Fund was created, on the initiative of the voluntary Committee on Endowment—organized in New York City in 1937—to insure the continuance of the JOURNAL OF DENTAL RESEARCH "in full accord with the highest ideals of strictly professional journalism devoted to the advancement of research," to which the Journal was dedicated at its establishment in 1919.

I. Amounts Received from Organizations
(May 22, 1945 to March 11, 1946)

Alpha Omega Fraternity	$25.00	
International Association for Dental Research—New York Section	25.00	
Louisiana State Dental Society	25.00	
Ohio State Dental Society—annual payment	60.00	
		$135.00

II. Amounts Received from Individuals
(May 22, 1945 to March 11, 1946)

$200: David Yurow (1 × $200)	200.00	
$100: Archie A. Albert, Lewis Fox, I. Hirschfeld, Frederick S. McKay, Arthur H. Merritt, Sidney Sorrin, Leuman M. Waugh (7 × $100)	700.00	
$50: William B. Dunning (1 × $50)	50.00	
$25: S. E. Davenport, Jr., Maurice William (2 × $25)	50.00	
$5: Joseph H. Kauffmann (1 × $5)	5.00	
$4: 50 F. A. Richmond (1 × $4.50)	4.50	$1,009.50
Total contributions 1945–46		$1,144.50

III. Interest, Income and Expense (Current Year)

No expenses were recorded.

Interest to date, March 11, 1946, on War Bonds:

Number of Bonds	*Denomination of Bonds*	*Date Purchased*	*Interest accrued during current year*
10	$1,000.00	March 1942	$160.00
22	1,000.00	May 1942	154.00
8	1,000.00	July 1942	104.00
2	1,000.00	December 1942	22.00
1	1,000.00	March 1943	11.00
1	25.00	February 1944	.17
5	1,000.00	March 1944	35.00
2	1,000.00	May 1944	6.00
1	1,000.00	July 1944	5.00
3	1,000.00	September 1945	....
2	1,000.00	March 1946	....
			$497.17

[1]Received for publication June 10, 1946.

Total contributions 1945–46 (Sec. I & Sec. II)	$1,144.50
Total addition to the Fund, including accrued interest for the current year	$1,641.67

IV. Summary of Total Assets: 1937–47

Total receipts (assets) for the Fund as of March 11, 1946	$42,214.39
Paid for 56 War Bonds (Sec. VI)	$41,440.00
Bonds and shares of stock (Sec. VI)	465.00
Balance	309.39

V. Cumulative Summary of Annually Recorded Items of Receipts and Expenditures

Year	*Contributions (payments)*	*Earned Interest*	*Expense*	*Net Receipts*	*Cumulative Annual Net Receipts*
1937–38	$10,652.50	None	$134.06	$10,518.44	$10,518.44
1938–39	4,748.17	None	None	4,748.17	15,266.61
1939–40	5,693.78	None	None	5,693.78	20,960.39
1940–41	5,190.55	$206.78	3.53	5,393.80	26,354.19
1941–42	4,166.35	333.95	None	4,500.30	30,854.49
1942–43	1,766.31	112.19	None	1,878.50	32,732.99
1943–44	3,302.23	334.00	None	3,636.23	36,369.22
1944–45	3,744.45	459.05	None	4,203.50	40,572.72
1945–46	1,144.50	497.17	None	1,641.67	42,214.39
	$40,468.84	$1,943.14	$137.59	$42,214.39	

VI. Statement Regarding Investment in War Bonds

By authorization of the Trustees of the Association, fifty-six War Bonds of Series F ("12-year appreciation bonds"), costing $740 each, have been purchased from the funds in the Endowment Committee's treasury, as of the following dates and serial numbers:

Purchased (10) March	1942: M140400F to M140409F, incl.	$7,400.00
Purchased (22) May	1942: M197811F to M197832F, incl.	16,280.00
Purchased (8) July	1942: M237558F to M237565F, incl.	5,920.00
Purchased (2) December	1942: M423990F and M423991F	1,480.00
Purchased (1) March	1943: M204356F	740.00
Purchased (5) March	1944: M750832F to M750836F, incl.	3,700.00
Purchased (2) May	1944: M752375F and M752376F	1,480.00
Purchased (1) July	1944: M892919F	740.00
Purchased (3) September	1945: M649868F to M649870F, incl.	2,220.00
Purchased (2) March	1946: M1061926F and M1061927F	1,480.00
		$41,440.00

The payee named on each bond is "Committee on the William J. Gies Endowment Fund for the Journal of Dental Research (an unincorporated body)." All of these War Bonds are in the custody of the Treasurer of the Committee, in a safety deposit box, Empire Trust Company, 580 Fifth Avenue, New York 19, N. Y., in trust for the Committee.

The contribution of $18.50, listed in Section II of the report for 1943–44 from Dr. Joseph E. Psayala (also mentioned in Section IV of this report), was in the form of a $25 United States War Bond Series F ("12-year appreciation bond"), Q631670F, the payee being "William John Gies Endowment Fund for the Journal of Dental Research." It is in the custody of the Treasurer in a safety deposit box, Empire Trust Company, 589 Fifth Avenue, New York 19, N. Y., in trust for the Committee. The bond was purchased in February 1944.

The Bethany Methodist Hospital Bond and Farnsworth Television and Radio Corpora-

tion Stock, indicated in Section IV, are also in the custody of the Treasurer of the Committee in a safety deposit box, Empire Trust Company, 580 Fifth Avenue, New York 19, N. Y., in trust for the Committee.

VII. Summary of Open Accounts Relating to Pledges

	Pledge	*Paid*	*Balance*	
A. Organizations				
Kansas: First District Dental Society	$100	$25	$75	
J. B. Mann Study Club, Washington, D. C.	1,000	200	800	$875.00
B. Individuals				
Harry Kaplan	250	100	150	
R. J. Rinehart	200	75	125	275.00
				$1,150.00

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II. International Association for Dental Research

Membership lists, including addresses of North American members and section rolls. This material is printed annually in the December number of the Journal of Dental Research.

Compiled by Edward H. Hatton, *Secretary-Treasurer*

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24. Budapest—Károly Balogh, István Bánhegyi, Béla Bonyhárd, János Brand, Béla de Simon, Károly Ferenczy, Dezsö Hattysazy, Oszkár Kömives, Ferenc Köszeg, Ervin Landgraf, Guztáv Morelli, Rudolph Rehák, Henrik Salamon, László Sugár, Steven Varga, Dénes von Máthé.—16.

25. Chengtu—Mary C. Agnew (Mrs. R. G.), R. G. Agnew, H. B. Collier, L. G. Kilborn, A. W. Lindsay, H. J. Mullet.—6.

26. Johannesburg—G. Friel, J. T. Irving, J. N. Noriskin, T. Ockersee, T. W. Osborn, J. C. M. Shaw, Julius Staz, W. A. Wilson.—8.

27. Iowa City—J. D. Boyd, A. W. Bryan, V. D. Cheyne, G. S. Easton, L. B. Higley, W. R. Kern, A. O. Klaffenbach, R. V. Smith, R. H. Volland.—9.

28. Detroit—Horton Kimball, S. J. Lewis, P. C. Lowery.—3.

29. New Orleans—A. K. Fisher, D. W. Lovett, H. W. Peterson, T. D. Speidel, S. L. Tiblier, F.J. Wolfe.—6.

30. Palestine—Heinrich Berger, Hugo Brasch, Ernst Kellner, Samuel Lewin-Epstein, Ervin Mansbach, Abraham Sussman, Jacob Yardeni.—7.

31. Houston—F. C. Elliott, Emmerich Kotanyi, F. A. Peyton, W. H. Scherer.—4.

32. Indianapolis—D. A. Boyd, G. T. Gregory, J. F. Hall, H. J. Healey, M. K. Hine, R. W. Phillips, H. M. Swenson, Grant Van Huysen.—8.

33. Tennessee—M. Don Clawson, Marguerite T. Dean, R. D. Dean, C. O. Dummett, J. T. Ginn, O. A. Oliver, C. R. Wood.—7.

Section membership—520, listed in 32 sections. Average membership per section—16.

Dental Materials Group[1]—J. R. Beall, S. F. Bradel, R. L. Coleman, P. F. Collins, I. D. Coy, W. H. Crawford, W. S. Crowell, Gustav Dahl, G. S. Easton, F. C. Elliott, A. H. Gabel, M. Goldberg, G. M. Hollenbeck, W. B. Holmes, E. J. Molnar, T. E. Moore, R. E. Myers, Clyde Nelson, G. C. Paffenbarger, F. A. Peyton, R. W. Phillips, C. E. Pomés, K. W. Ray, I. C. Schoonover, J. S. Shell, E. W. Skinner, Wilmer Souder, J. T. Sweeney, W. T. Sweeney, N. O. Taylor, P. B. Taylor, S. D. Tylman, Raymond Vines, M. L. Ward, E. M. Wise, H. K. Worner.—35.

Residents of North America, but not now members of a Section—Frank Allen, Jr., S. R. Atkinson, S. N. Blackberg, P. E. Blackerby, J. L. Bradley, J. C. Brauer, R. E. Brawley, J. C. Brown, Marcu Brucker, C. G. Burn, C. P. Canby, R. H. Coleman, P. F. Collins, K. H. Cross, P. P. Dale, W. M. Davis, C. F. Deatherage, R. W. Edwards, L. L. Eisenbrandt, S. B. Finn, B. Gottlieb, E. R. Granger, Gustav Haber, H. L. Hansen, L. P. Hartley, H. E. Harvey, R. L. Hayes, W. B. Holmes, B. L. Hooper, A. O. Hubbell, E. A. Jasper, Oscar Kanner, D. A. Keys, W. F. Koss, Melvin Land, V. E. Levine, A. W. Mann, J. B. Mann, J. F. McClendon, F. S. McKay, A. L. Midgley, E. J. Molnar, T. E. Moore, F. W. Morse, Jr., C. A. Nelson, Anna C. Nichols, H. J. Noyes, Mathew Podolin, V. H. Powell, H. E. Raper, C. V. Rault, B. T. Ritchey, M. I. Schamberg, H. M. Schamp, E. O. Scott, H. J. Sedwick, N. F. Simmons, F. V. Simonton, J. A. Sinclair, Margaret C. Smith, J. T. Sweeney, A. P. S. Sweet, M. L. Tainter, Edward Taylor, N. O. Taylor, W. C. Taylor, R. F. Vines, G. D. Wessinger, G. R. Winter, Edmund Wise, H. B. Younger.—71.

Not residents of North America and not now members of a Section—J. L. Boots, Fernando Lara Brava, Ove Brinch, C. D. M. Day, Gustav Dahl, Jorge Erausquin, Guido Fischer, Humberto Granados, Walter Hess, R. E. T. Hewat, J. J. Holst, G. Y. Hildebrand, Frode Hilming, H. F. Humphreys, T. E. de Jonge-Cohen, Gustav Korkhaus, G. D. McPhee, Maurine W. Müller, Pekka Nironen, Ch. F. L. Nord, T. Ockersee, Silvio Palazzi, P. O. Pederson, Paul Pincus, Carlos E. Pomés, Friedrich Proell, F. M. Pucci, Inghold Reichborn, Elkan Sanders, J. C. Sartori, Reidev Sognnaes, Guttrom Toverud, Robert Weaver, Rudolph Weber, J. J. Wolfe, H. K. Worner.—36.

Total membership as of Nov. 10, 1946—627.

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[1] Members of the Materials Group may and in most instances do belong to Sections.

III. BOOKS RECEIVED, 1946

Accepted Dental Remedies, 12th Ed. (Chicago, American Dental Association: 1946) pp. 316.

American Dental Association, Roster of Members (Chicago, Am. D. Assoc., 1946) pp. 343.

Burket, L. W. *Oral Medicine* (Philadelphia, J. B. Lippincott Company: 1946) pp. 674.

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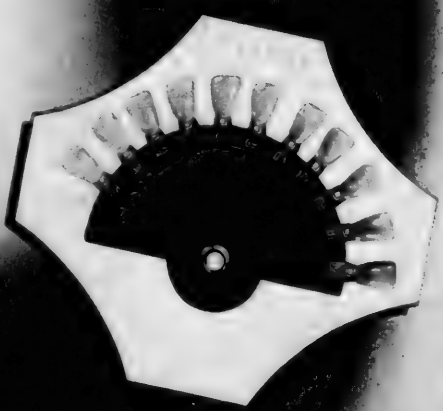

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